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Reanalyzing the Activity Anorexia Protocol Using an Addiction Theory

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REANALYZING THE ACTIVITY ANOREXIA PROTOCOL

USING AN ADDICTION THEORY

A Thesis

by

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ABSTRACT

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One researched and developed phenomenon is activity anorexia (AA) in rats and its resemblance to the human pathology anorexia nervosa (AN). Researchers have long relied on this comparative model for AN as AA. This study aims to reanalyze the overt physiological phenomena of AA to test an addictive theory against the prevalent AA theory. Equal numbers of subjects could eat or run during a one hour feeding period and all activity was monitored per 30 minutes. Results showed that daily running correlated with an increase in food consumption with no effect of food suppression. The only factor that regressed to food or activity was days in the protocol. An anticipatory response was elicited and the animals would run excessively and sporadically whenever their exhaustion had subsided. It is proposed that the observed running behavior and activity may be better explained by addictive behaviors rather than an anorexic phenomenon.

DEDICATION

I want to dedicate this to my parents, Pedro and Rosa Maria Botello, and my brothers, Jesus and Martin. Without their limitless support and affirmation, none of my aspirations would meet fruition. To my brothers, whose intellect and affection encouraged me to be a better scientist, I appreciate your support.

“El fin del mundo será decidido por aquello que no exploramos.”

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Lastly, to my shepherd, who sat unwaveringly as I completed this project even though he wanted to play fetch, thanks for the company. That’s a good boy, Titan.

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CHAPTER I

INTRODUCTION

The reproduction of human behaviors in animal models is a hallmark of psychological studies that implies universal behaviors. Many seminal researchers use of various animals to reproduce humanlike responses based on the theory of conditioning or learning has allowed researchers to develop protocols to study relationships between animal and human behavior. These specific procedures elicit certain responses that allow psychologists to study behaviors, instincts, and mechanisms. Take Harry Harlow, who observed the choice of infant rhesus monkeys to cling to a terrycloth surrogate mother instead of the wire feeder surrogate. He concluded that infants preferred the comfort over the necessity of food due to an underlying psychological need (Harlow, 1958). Harlow's conclusion was generalized to the need for nurturing factors among natural pressures within human infants. Alternatively, the importance for emotional support of attachment in infants' developmental processes, as it more commonly known (Bowlby, 1958). It is classic examples of Harry Harlow or Ivan Pavlov's seminal work with dogs, that helped to create the theory of classical conditioning, that exemplify the importance and influential work comparative studies can produce.

B.F. Skinner's contributions to the field and use of the theories of conditioning and learning to establish behavior analysis was furthered by behaviorists to treat human problem behaviors (Morris, Smith & Altus, 2005). These ideas led to the production of applied behavioral analysis (ABA) treatment of human pathologies by way of behavior modification (Morris et al.,

2005). In ABA, the therapist will functionally analyze the client's problem areas to develop an idiosyncratic treatment plan that utilizes behavioral interventions to treat the client's problem behaviors (Kirk, 1999). ABA treatment style utilizes multiple strategies (e.g. shaping and modeling) and exercises (e.g. role playing and systematic desensitization) to modify the client's behavior. This systematic treatment has been expanded to treat attention deficit – hyperactive disorder (ADHD), autism, neurodevelopmental disorders, and even conduct disorder (Evans, Scotti & Hawkins, 1999). ABA practitioners may apply the theories and effects discovered and employed in comparative psychology studies to their clients (Pierce & Epling, 1994).

Following the same path of application, modern use of animal protocols attempts to develop behaviors similar to human pathologies for the development of treatment or further investigation that would be difficult with human participants (e.g. hormonal levels). For example, a protocol named learned helplessness used with dogs, initially, and with rats, goldfish, cats, and mice elicits similar behavioral outcomes as observed in human depression (Seligman, Rosellini, & Kozak, 1975; Hiroto & Seligman, 1975). The dogs or rats are exposed to sessions of inescapable electric shock to the point where the animal ceases to attempt escape when given an option to. The use of this animal protocol and experimentation lead to the development of learned helplessness as the underlying factor for human depression, because people displayed similar behaviors and experience patterns (Seligman, 1975). Today, the learned helplessness theory of depression has been revised, but continues to be utilized to develop pharmacotherapy and behavioral treatments for depression (Rehm, Wagner & Ivens-Tyndal, 2004). Other psychopathologies with animal models include schizophrenia (Lyon, 1991a), mania (Lyon, 1991b) and ADHD (Feldon & Weiner, 1991).

Naturally, research on human participants usually remains at a quasi-experimental level, but animal studies allow for more rigorous experimentation. Initial use of electric shock with human participants would have been impractical in the development of the phenomenon of learned helplessness. Comparative psychology allows for an avenue for research to be conducted on pathologies that are dangerous or unethical for usage with human participants (Shapiro, 1998; Willner, 1991). Furthermore, it allows for more controlled methods in an experimental fashion. There are some fundamental controversies in theory of utilizing animal models. The applicability of information gathered from animal experimentation is limited, but continues to show promising results in human transference (Shapiro, 1998). Certainly, there is also a concern about the clinical use of the animal-developed treatments (Willner, 1991). For the biomedical and pharmaceutical field, animal studies have proven useful prior to human trials (Shapiro, 1998). For mental health, behaviorism and ABA have proven animal models' worth (Pierce & Epling, 1994).

One such pathology that is difficult to study is eating disorders, especially anorexia nervosa (AN). Eating disorders, including both AN and bulimia nervosa, have the highest mortality rate among psychiatric disorders (Arcelus et al., 2011). According to the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5), AN is characterize by restricted caloric intake, low body weight, fear of gaining weight, weight reduction behaviors, and a disturbance in one's self-body image (American Psychiatric Association [APA], 2013). The debilitating nature of anorexia nervosa on the person's physique and psychology make it difficult to study or experiment. With a modern point prevalence rate of 0.2 - 2% of eating disorders in the population (Williamson et al., 2004), gathering a sufficient clinical sample size for treatment experimentation makes it more difficult for clinical researchers. The utility of an

animal model for AN or an eating disorder, in research terms, would be high. It would allow various experimentation to be conducted on treatment options for these debilitating pathologies.

Through replication and research, an animal model, commonly referred to as activity anorexia (AA), was established that elicits similar behaviors to anorexia nervosa in humans. Generally, rats are restricted food access to 1-3 hours a day and given unlimited access to an activity wheel (Gutierrez, 2013). Rats will progressively engage in a high level of activity, gradually reduce body weight, and increase eating before stabilizing food consumption. Depending on the protocol's procedure, the rats will enter a dangerous level of running and food consumption that could lead to death by inanition within 10 days (e.g. Routtenberg and Kuznesof, 1967; Pierce & Epling, 1994). The rat's running and feeding will increase throughout the protocol, but their feeding will be much less than controls (Routtenberg & Kuznesof, 1967). It is the phenomenon of these experimental rats consuming less than their control counterparts that many researchers point to as one of the prevalent pieces of evidences for AN in the rats, but many other characteristics are still present. Some researchers refer to this trend as "self-starvation" in the rats (Routtenberg & Kuznesof, 1967); others see it as running interfering with eating habits (Pierce and Epling, 1994). Overall, the marginal difference in food consumption and extreme weight loss inspires many researchers to view it as an anorexic effect. Many theories have emerged for explaining the phenomena, but there is no consensus.

The intent of this project is to further explore the understanding of this phenomenon and review the presented theoretical underpinnings. An addiction theory will also be incorporated in the theoretical review to help explain the mechanisms by which the rats' behaviors are elicited. Using advancements in technology, rats' activity and inactivity will be reviewed intensively on an every 30-minute basis. Furthermore, this study attempts to assess the preference of rats to run

or feed during the feeding hour. It is hypothesized that the increase activity observed in the literature is better understood by an addiction theory.

CHAPTER II

INITIAL RESEARCH

Hall and Hanford (1954; Hall et al., 1953) first reported the phenomenon of rats developing increased running habits when allowed to run on a feeding-restricted schedule, but they did not further investigate the food consumption habits. Their initial focuses were on the drive that hunger produced and whether drive increased as the organism continued through a food-deprivation schedule. Through these experiments, they concluded that drive undoubtedly increased as evidenced by the 1400% increase in activity as compared to base-rates (Hall et al., 1953; Hall & Hanford, 1954). In other words, the hunger drive the rats experienced while on the food-deprivation schedule was not static, but increased as evidenced by the running. Reid and Finger (1955) concurred with Hall et al. (1953) that the hunger drive experienced by the rats continues to increase as the days progressed. Reid and Finger (1955) also found that the rats maintain their high activity levels until, at minimum, 15 days after the 23-hour cycles have begun. They concluded 15 days was the threshold the rats needed to adjust to the food-deprivation. While the daily activity of the rat seemed to stabilize past the 15 days, the rats still showed increased activity post-feeding period. Reid and Finger (1955) speculated that the rats might still be adjusting to the schedule up until the 35th day. Strong (1957) questioned whether the apparatus used in the collection of activity added to the observed activity. He also found that hunger increased activity, and that certain apparatuses were more suitable for the food-restriction

schedule. The general trend of the phenomena when it was originally studied focused on the increased activity.

A new caveat to the literature that Reid and Finger (1955) contributed was the daily study of rat weight and food consumption. They noted that the rats continued to decrease in body weight and reached an average of 31% decrease compared to baseline measures. The full trend showed a 5% increase during baseline, a 31% decrease during food-deprivation, and an increase back to a 13% decrease during recovery when compared to initial weight base-rates (Reid & Finger, 1955). As for food consumption, their rats slowly increased food consumption before reaching a limit of 60% of base-rate during food-deprivation. The only outstanding trend they noted was that the rats began restricting their water consumption during food deprivation. This was one of the first studies to acknowledge a reduction in rat weight and a limitation to food consumption by the rats.

Of further interest, Hall and Hanford (1954) argued that their data did not indicate a "satiation syndrome" that Reid and Finger (1955) had suggested they observed. This proposed syndrome was a drastic drop in activity following a recovery phase, indicating that the rats lost substantial hunger drive in running once they returned to an ad libitum feeding. Reid and Finger (1955) observed that the rats dropped to 61% of base-rate in activity on the first day of recovery. After that, activity rebounded and stabilized around base-rate levels. A preliminary reversal of the activity levels would have been to satisfy the hunger drive.

In the zeitgeist of the time, the researchers of the first food-deprivation schedules in rats were more focused on studying the psychodynamic drive of hunger. These researchers did not further explore marked drops in rat-body weight and limited replication of the phenomena was completed in this time. It was also not their intention to compare this effect to any pathology of

their time. “Anorexia nervosa” was published in the first edition of the DSM in 1952 as a specifier to psychophysiologic gastrointestinal reaction (APA, 1952). However, the disorder, including other eating disorders, did not gain popular recognition in the masses until the 1970s and 1980s (Deans, 2011). AN was known in the clinical settings, but was a rare syndrome to encounter (Dean, 2011). The AA protocol lay dormant in the literature until Routtenberg and Kuznesof (1967) began reusing it to test for nonadaptive behavioral patterns. They first identified the “self-starvation” pattern, but it would be more than twenty years later that researchers noticed the similarity between the human pathology of AN and the rat phenomena of AA (Shapiro, 1998). Since then, the theories have changed as the information regarding both AN and AA have progressed.

Inability to Adapt

Routtenberg’s and Kuznesof’s (1967) research involving positive incentive mechanisms led them to study the effect food deprivation and activity had on weight maintenance. In their earlier experiments, rats continued to self-administer a pleasure electrode over eating and would eventually die from starvation (Routtenberg & Lindy, 1965). Their focus was to induce a similar nonadaptive pattern of behavior through external stimuli. They found that the restriction of food and access to a running wheel produced lower food consumption and increased weight loss on experimental subjects compared to controls (Routtenberg & Kuznesof, 1967). Upon further investigation, Routtenberg and Kuznesof (1967) found that the length of feeding period and familiarity of apparatus significantly affected the rats’ performance. They also found that chlorpromazine reduced the difference in weight loss and food consumption between control and experimental groups, and lowered activity. They noted that chlorpromazine had been used, at their time, with clients dealing with anorexia nervosa to increase food intake (Routtenberg &

Kuznesof, 1967). It was hypothesized that chlorpromazine worked to interfere with the pleasure mechanism that activity stimulates. With these experiments, Routtenberg and Kuznesof had repeated the "self-starvation" effect and found that chlorpromazine could treat it.

Upon review of these experiments and furthering his research, Routtenberg (1968) proposed convergent stressors were integrating to produce the observed effects. He stated that the rats were not adequately adapting to the food-deprivation schedule and new environment causing them to experience a "novelty stress" (Routtenberg, 1968). In other words, the transfer of the rats to the activity apparatuses stressed the rat aggravating the deprivation effects. The primary stressor, being "deprivation stress", accounted for the effects of the food-restriction schedule on activity and weight-loss. Routtenberg acknowledged that both stressors play major roles in the observed phenomena, but he hypothesized the underlying process may be protein catabolism (Routtenberg, 1968). The experienced stress might affect the corticoid mediation of gluconeogenesis causing hypoglycemia. As this condition persists, it would satiate the rat to its feelings of hunger by increasing adrenocortical activity, which would affect the hypothalamus. To be more specific, the hypothalamus-pituitary-adrenal axis (HPA) is activated during hyperactivity causing a decrease in hunger sensation (Duclos, Gatti, Bessiere, & Mormede, 2009). Overall, Routtenberg proposes that the stress levels will satiate the rat to feeding leading to the nonadaptive behavior of self-starvation.

Dwyer and Boakes (1997) revisited this theory in their review of what they called "activity-based anorexia". Following Routtenberg's (1968) conclusions about novelty stress, they attempted to preadapt the rats to the feeding schedule and activity wheel. Through a few experiments, Dwyer and Boakes (1997) found that the time of feeding, preadaptation to the schedule, and running time could act as protective factors for the rats developing activity-based

anorexia. Most noticeable was the feeding of the rats during the night-cycle and not allowing them to eat post-running allowed the rats to adapt to the protocol. Rats are naturally nocturnal eaters; therefore, the animal was better at responding to the protocol by providing the food during the dark cycle. Allowing a rest period between running and eating, by denying access to the wheel, significantly reduced weight loss. Dwyer and Boakes (1997) suggest the relationship between activity, body weight, and food consumption in AA is not direct, and that prior adaptation can prevent this phenomenon. Their research shows that rats are failing to adapt to the feeding schedule, but other processes may be affecting the animals.

These researchers brought about a new focus to the AA world in which the focus was on the animal's weight loss and the relationship activity may have on it. Naturally, the logic is that activity should be governed by the amount of caloric intake. If there is no energy to expend, then the rats should not engage in much activity. Routtenberg and others observed the rats' running regimen and the nonadaptive effect it was producing. The stress theory Routtenberg (1968) proposed was the first attempt to explain the possible relationship. Using his dual stressors, he suggested that a more neurological effect must be the underlying cause. Thus, the rats' running was indirectly affecting the self-starvation by way of corticoid catabolism. Dwyer and Boakes (1997) support this idea of adaptive difficulty. They found that certain procedures could significantly increase the rat's resiliency to developing activity-based anorexia and its survival. By increasing the rat's adaptive abilities to the AA protocol, the mortality rate and weight loss were reduced.

These researchers' hypotheses explained much of the rat's behavior, but some limitations are present. For example, it fails to explain what causes the rats to continually increase in activity. The deprivation stress would need to continue past the first few days and increase as

well. A continuous level of stress would not lead to a progressive increase in activity, but the rats' running levels would find a plateau relative to the stress. He is unclear about the effect the primary deprivation stress would have on the rats' activity or the trend in stress levels during the protocol. They would need to rely on Hall's and Hanford's (1954) conclusions about hunger drive to answer the question regarding deprivation stress levels. Alternatively, the deprivation schedule would cause an accumulating effect on the stress leading to a higher level the next day. Secondly, the processes involved in activating the HPA and affecting food consumption is never fully explained. Even recent researchers cannot explain the exact nature of the HPA's involvement in AA (Duclos et al., 2009). It is known that stress levels do affect the subject's activity (Duclos et al., 2009). Nevertheless, speculation about the rat's stress levels during this protocol is problematic to quantify, furthermore study. Another researcher considered understanding the rats' behavior from another stance and drew semblances to a human pathology.

CHAPTER III

ANOREXIA NERVOSA COMPARISON

Pierce and Epling (1994) proposed a second theory to explain the trends of activity levels rising and food consumption decreasing in the AA protocol. They posited there is a direct relationship involved in which the activity of the rat was competing with its feeding. To explain further, the rat's activity was causing an imbalance leading to the rat's inability to consume sufficient and sustainable amounts of food. Unlike Routtenberg's theory, Pierce and Epling (1991) do not rely on stress to explain the development of AA. They propose that AA is the result of an interplay between evolutionary forces, behavioral processes, and physiological process. In their analysis of these processes and their relation to the development of AA, they provide evidence of the similarities between AA (or as they commonly refer to it as activity-based anorexia) and AN. Furthermore, they argue that many cases of AN are better explained using AA symptomology.

As Pierce and Epling (1991, 1994, 1996) present it, the food deprivation is the primary initiator in the process of AA. In times of food scarcity, many animals will engage in anorexic behaviors and hyper/hypoactivity (Mrosovky & Sherry, 1980). These species adapted to the pressures based on the characteristics of their environments by developing hypo- or hyperactive behaviors. In some cases, hypoactivity like hibernation led to a higher survival rate, while others' hyperactivity increased their chances of survival. In rats, natural selection possibly favored those who ran to search for new food. By providing the artificial food scarcity schedule, the rat

naturally begins to become more active in its pursuit of a new food source. The wheel's availability in AA provides the stimulation the rat needs to satisfy this instinctual hyperactivity, but this evolutionary process is not the singular factor involved in developing AA.

Running has reinforcing properties (Pierce & Epling, 1991; 1994; Epling & Pierce, 1996; Belke, 1996; Perez-Padilla et al., 2010; Duclos et al., 2013; Giel et al., 2013). Taken as itself, running can be a rewarding factor by activating endogenous opioids (Belke, 1996) or dopamine (Bergh & Sodersten, 1996). With sufficiently intense exercise, many people have reported experiencing a surge in endorphins known as the "runners high" (Powell, Honey & Symbaluk, 2013). Once the rat experiences this surge in neurotransmitters, it will urge it to run more as it is a rewarding feeling. Furthermore, running, when combined with food restriction, produces a suppression reaction for appetite through those same neurotransmitters (Pierce & Epling, 1991, 1994). Not only is the running positively reinforcing itself, it is being negatively reinforced through appetite suppression. This dual action reinforcement for running exaggerates the initial push by evolutionary forces and creates a sort of slippery slope for the animal (these processes are explained below as a more in-depth analysis of the role neurotransmitters play in AA is examined).

Pierce's and Epling's behavioristic take on AA revolutionized the field's theories, but it was their postulation of the resemblance between AA and AN that made researchers refocus. By studying the similarities between the two phenomena, they proposed that most AN cases are AA cases present in human patients (Pierce & Epling, 1991; 1994). Both phenomena do not simply elicit a self-starvation behavior or drastic changes in body weight, but share similar physiological characteristics. They also argue that the two follow a similar developmental pattern.

Primarily, hyperactivity is a major factor involved in both AA and most cases of AN (Pierce & Epling, 1994). They found that many cases of AN also started with some form of activity increase such as running. This increase in activity also accompanied a marked drop in appetite (Pierce & Epling, 1994). Many patients reported reducing food consumption shortly after starting an intense activity regimen (Wheeler, 1996). Adding to the relationship between running and consumption, food intake returns to a static state after exercise is reduced (Epling & Pierce, 1989). They argue that research has shown that lowered caloric intake in humans can also increase lead to an increase in activity. They report that it is necessary for many individuals to have started reducing food intake and increasing activity to begin experiencing the symptoms of AN. Lastly, they point to the convergent evidence of the disruption of the estrous cycle in female rats and anorexic patients that indulge in high levels of physical activity (Pierce & Epling, 1991; 1994). Both, a reduction in food consumption and increase in exercise, are necessary to elicit the phenomena of AA. It is these factors that Pierce and Epling (1991; 1994) argue are necessary in most cases of AN as well.

To fully judge the comparability of AA and AN, one must review the diagnostic criteria of AN to the noted symptoms of AA. Anorexia nervosa's DSM-5 criteria includes the following symptoms:

> "(A) Restriction of energy intake relative to requirements, leading to significant low body weight in context of age, sex, developmental trajectory, and physical health … (B) Intense fear of gaining weight or becoming fat, or persistent behavior that interferes with weight gain … (C) Disturbance in the way in which one's body weight or shape is experienced"
>
> (APA, 2013, pp. 338-339; Appendix A)

Many of these symptoms can be relatively compared to AA in rats, but there is difficulty explaining all of them. The restriction of food consumption is unequivocally present in AA, but the other two criteria are not as clear. Naturally, the fear of a rat cannot be quantified or described simply by observing the rat. Certain physiological markers may indicate a state of heightened stress, but to know whether the rat feels fear or is actively trying to lose weight is absurd to attempt to distinguish. The second part of that criteria B is a continuous pattern of behaviors that propagate the weight loss. This the rat does exhibit by continuously increasing wheel running until inanition forcibly reduces it. The third criteria, C, indicates a cluster of behaviors that deal with self-body image and the distorted perception anorexic patients will describe themselves. Once again, it would be ridiculous and impossible to quantify a rat's self-body image. Pierce and Epling (1994) argue that as societal pressures lead to the distorted self-body image that AN patients experience, the forced food deprivation substitutes for that pressure. It is based on the philosophical difference between free will and determination. The person did not 'choose' to have the distorted view of him- or herself; their environment pressured them into it through social learning. Likewise, the environment pressures the rat into the food deprivation schedule. By proxy of the schedule, Pierce and Epling argue that the rat develops and meets the humanistic AN criteria in the DSM-5.

These correlations and trends between human and animal AA do not suffice in explaining the mechanisms or processes involved in either AA or AN. Past and present studies have geared more towards the role endogenous opioids have in this phenomenon. More specifically, focus is placed on the HPA to explain the neurological involvement in producing AA. Other physiological systems are reviewed for their convergent evidence in these phenomena. From these studies, researchers also attempt to develop treatments for AA and AN.

Effect on Neurotransmitters and Hormones

Many researchers have speculated the effect AA is having on neurotransmitters and hormones within the rats is through the HPA (Routtenberg, 1968; Pierce & Epling, 1994; Duclos et al., 2009) and the hypothalamus-pituitary-gonadal axis (HPG; Pirke, 1996). The HPA is an important structure in the process of responding to stress from external stimuli (Spencer & Deak, 2017). It is hypothesized that the food deprivation and activity cause enough stress to temporarily damage the HPA and effect multiple hormones and neurotransmitters (Routtenberg & Kuznesof, 1968; Pirke, 1996). Likewise, AA is predicted to affect the HPG (Pirke, 1996) which mediates the hormonal balance between the hypothalamus and gonadal glands (Meethal & Atwood, 2005).

Many psychological disorders and conditions have been associated to dysfunction in the HPA axis (Spencer & Deak, 2017). The main process of the HPA is regulation of glucocorticoid, or corticosterone in animals, which many refer to as the "stress hormone" (Spencer & Deak, 2017). It is theorized that the food deprivation schedule causes an increase in stress levels leading to the persistent hunger drive (Routtenberg, 1968; Pierce & Epling, 1994). The continued deprivation leads to a constant state of stress that will dysregulate the HPA. While this does not lead to a continual increase in corticosterone levels, the chronic stress can cause frequent increases in hormonal levels in the HPA (Spencer & Deak, 2017). In one study, corticosterone levels in male rats were measured prior and during the administration of a food restriction protocol, and compared to control rats. The researchers found that the deprivation alone raised corticosterone levels, and activity and deprivation produced a much higher level of corticosterone (Pirke, 1996). From similar studies, it is generalized that the HPA is involved in AA (Pierce & Epling, 1991). Activity can influence the HPA, but the neural pathways or

structural pathways that lead to this effect are unknown (Spencer & Deak, 2017). One criticism is that glucocorticoid is a wide spread hormone that is present throughout the body and can traverse the blood-brain barrier which makes knowing all its effects difficult (Spencer & Deak, 2017). The role HPA plays in the AA phenomena is uncertain, but there is evidence of it being affected. The combination of starvation and exercise can affect many systems, and the HPA is not immune to it.

The HPG's function in the AA phenomena makes up a large part of the convergent evidence for AN as an AA effect. The main function of the HPG is to regulate sex steroids and by proxy, estrogen, testosterone, progesterone and inhibin (Meethal & Atwood, 2005). It is the primary axis for which sexual development is regulated by way of the androgens or estrogens, respective to sex (Meethal & Atwood, 2005). The HPG works on a cycle. The hypothalamus will secrete gonadotropin-releasing hormone (GnRH) which signals to the pituitary to release luteinizing hormones (LH). The LH will signal the gonads to release activins and androgens/estrogens. The androgens and estrogens will signal, in a negative feedback loop, to the hypothalamus to stop secreting GnRH. Once the androgens or estrogens are below a threshold, the hypothalamus will begin secreting GnRH. Disruption or dysregulation of the HPG will lead to an imbalance in the sex hormones; disruption of these hormonal levels in the HPG will affect other processes and systems. In AA, the high activity levels and low weight disrupt a female's estrous cycle (Pierce & Epling, 1991; 1994; Gomez & Martinez Sanchez, 2013). In a study involving male rats, LH and testosterone suppression was observed in an AA protocol (Pirke, 1996). Both rat sexes showed a dysregulation of the HPG, but the estrous cycle has more research on it.

Female rats and humans provide an important comparative case for AA. As discussed earlier, females are more sensitive to changes in the HPA (Spencer & Deak, 2017) and HPG (Meethal & Atwood, 2005). Furthermore, AA does cause a disruption in the estrous cycle akin to the disruption of the menstrual cycle for anorexic women. Pierce and Epling (1991) attribute these disruptions in sexual functioning to the high activity that both AN and AA will produce. They state that female athletes, especially marathon runners, will also experience affected menstrual cycles (Pierce & Epling, 1991). As the person's, or rat's, body weight and exercise reaches a critical point, the menstrual cycles are disrupted. Although body weight is the more crucial factor for affecting the HPG (Pirke, 1996), Pierce and Epling (1991) argue that it is due to the increased exercise that the body weight meets that threshold. Pirke (1996) reported that a study showed that the combined effects on hyperactivity and food deprivation, lead to a faster disruption of the menstrual cycle. Hyperactivity is involved in the effects observed on the HPG, and weight may be a mediating factor to that effects path.

β – Endorphins

While the HPA and HPG are the theorized pathways for the effects seen in AA, the major neurotransmitters proposed involvement are endorphins (endogenous opioids). Both HPA and HPG axes affect numerous neurological and physiological systems directly and indirectly. It is difficult to single out the processes that are involved in AA, but studies have noticed trends involving endogenous opioids. More specifically, researchers have studied the effects β-endorphins have.

Endogenous opioids, such as β-endorphins, have a highly rewarding effect due to their addictive and pain reducing qualities. β-endorphins are released during hyperactivity (Colt, Wardlaw, & Frantz, 1981; Pierce & Epling, 1994; Belke, 1996), which increases its levels. As

explained earlier, these endorphins work as a positive and negative reinforcer during the AA protocol to strengthen running behavior. As the organism reaches the "runner high" point of running, the body may develop tolerance to the increased levels of β-endorphins (Pierce & Epling, 1994). The interaction of the reinforcing properties of β-endorphins and tolerance to them would cause the rat to progressively exercise more intensely. Not only are the β-endorphins suppressing appetite, Pierce and Epling (1991; 1994) contest that they, in fact, compete against one another.

The hyperactivity may increase β-endorphins levels, but the food deprivation may also be involved. It is theorized starvation increases corticosterone in the organism (Routtenberg, 1968; Pierce & Epling, 1994). This continued stress will then cause a dysregulation in the HPA, which leads to a continued drive to exercise. The increased exercise, increased β-endorphins levels, and decreased body weight through food deprivation interact to disrupt the HPG (Pierce & Epling, 1994; Geer & Warren, 1996). It is this process that may explain the resulting dysregulation of the estrous and menstrual cycles observed in AA and AN, respectively.

Application in Clinical Settings

As stated previously, some research done with animals is attempted to be transferred to human patients. Many researchers have found reversal effects in AA that may have implications for patients with AN. Furthermore, pharmacotherapies and behavior modifications are being developed using the AA to treat certain behaviors, such as: feeding schedules (Dwyer & Boakes; 1997; Pierce & Epling, 1991), dieting (Brown, Avena & Hoebel, 2008; Powell, Honey & Symbaluk, 2013), exercise (Gutierrez, Cerrato, Carrera & Vazquez, 2008; Cerrato et al., 2012), and weight gain (Hillebrand et al., 2005).

As discussed earlier, Dwyer & Boakes (1997) found that changing the feeding time would help protect the rats from developing AA. They also found restricting the running times helped to reduce weight loss and increase caloric intake. Pierce and Epling (1991) noted that splitting the feeding time into multiple periods allowed the subjects to adapt better to the schedule. Changing the times the rats ate significantly affected the phenomenon of AA. Eating small meals throughout the day, as opposed to one restricted eating period, could assist those in maintaining a healthy process.

Another suggestion the AA protocol has led to in human application is the limitations to dieting and exercise. Studies showed an interaction effect of food restriction and activity that leads to the marked drop in weight (Pierce & Epling, 1994). It is recommended that athletes, dancers, or models who maintain a certain high exercise regimen and diet need to be vigilant to not develop AA or AN symptomology (Powell, Honey & Symbaluk, 2013). This is especially geared for those who need to maintain a certain weight, like boxers. Another protective factor is to begin dieting before initiating an exercise program. Dwyer and Boakes (1997) found that animals preexposed to the feeding schedule were less likely to develop AA when the activity wheel was introduced. While this interaction effect can be advantageous for those who desire to lose weight, it is possible to slip into the clinically significant range. Research has also shown that changing the nutritional characteristics of the food can also reduce AA symptomology. Brown, Avena, and Hoebel (2008) found that a high-fat diet with or without sweetness can reduce self-starvation and weight loss. One of the frequent treatments for AN, whether inpatient or outpatient, is to focus on dietary habits and regain the proper nutrients or feeding schedules (Williamson et al., 2004). Adhering to a diet and/or exercise regimen is effective in reducing weight, but if it becomes too restrictive or intense AN could be induced.

Some interventions geared towards the exercise portion of the phenomena have been suggested. In AA, the animal will also experience a significant drop in internal temperature and may prefer a het pad to running (Gutierrez et al., 2008). Over multiple experiments, Gutierrez et al. (2008) and Cerrato et al. (2012) found that increasing the ambient temperature of male and female rats reduced activity and increased weight and food consumption. They suggest a similar treatment for patients with AN could reduce the hyperactivity many of them experience. Increasing the ambient temperature during impatient settings could reduce activity and increase caloric intake (Cerrato et al., 2012). Furthermore, the heat could reduce the caloric expenditure of patients who naturally need to produce more heat. Through reduced body weight, anorexic patients have a low body fat and are unable to regulate properly their homeostatic temperature process (Gutierrez et al., 2008). The authors argue that heat can make a difference, but other factors may be involved. They also note that this effect does not address the cognitive and emotional aspects of AN, but is only a behavioristic intervention through environmental modification (Cerrato et al., 2012).

Pharmacotherapies are another avenue researchers have explored. Routtenberg and Kuznesof (1967) found that chlorpromazine reduced the difference between control and experimental groups in terms weight loss and food intake. They note that previous studies have shown that chlorpromazine can increase food intake in anorexic patients (Routtenberg & Kuznesof, 1967). Hillebrand et al. (2005) studied the effect olanzapine could have on AA rats. The researchers were drawn to this pharmacotherapy because olanzapine had been shown to increase body weight in humans (Hillebrand et al., 2005). Hillebrand et al. (2005) found that olanzapine: prevented the rats from developing a lower body temperature and reduced HPA activity in AA rats; increased weight and caloric intake in ad libitum feeding rats; and reduced

activity in anorexic patients that had hyperactive symptoms. The authors suggest that olanzapine and other HPA affecting agents should be further explored as possible treatments for AN.

Limitations to Transference

Despite all the reviewed literature, the actual effect endorphins have on AA is disputable and the actual role the HPA and HPG axes play are equivocal. Definitive evidence for the effect the neurotransmitters and hormones have on AA is not presentable. As mentioned by Spencer and Deak (2017), the HPA affects many systems and physiological mechanisms. Even in Hillebrand et al.'s (2005) analysis of the effects of olanzapine, they conclude that the actual systems affected by it are uncertain. Pirke (1996) points out that the physiological aspects of a rat's neurology are too different from a human's process for the transference of pharmacotherapy to be easily applicable. Pirke (1996) suggests that effects on activity should be the only ones to be focused on. Another limitation is that AA treatments cannot account for cognitive aspects that are present in AN (Cerrato et al., 2012). Clinical application of the information provided by this research into appetite and running has been slow to transfer. Most of it is theorized, but has yet to be tested. Behavioristic interventions seem to be the only ones making a difference in anorexic clients (Williamson et al., 2004).

CHAPTER IV

UTILIZING AN ADDICTION THEORY

Another theory remains semi-dormant in the literature in which researchers view the increased running levels as a dependency. It was originally proposed by Marrazi and Luby (1986) that the phenomena in question was a result of addiction to endogenous opioids. Following the same evidence as Pierce and Epling (1994), they theorized that the running and self-starvation are simply a reaction to the overflow of opioids during intense activity. Pierce and Epling, however, do not rely on the reinforcing attributes of running to explain the whole phenomena. They posit the running is only a part of the grand scheme of the AA syndrome that includes food deprivation and competing reward systems. Marrazi and Luby (1986) hypothesize that the behaviors observed in AN are better explained by an addiction model. They state that the endogenous opioids secreted during hyperactivity provide a better explanation for the phenomena and do not have to attempt to solve the cognitive question involved in AN (Marrazi & Luby, 1986).

Much like the processes proposed by Pierce and Epling (1991; 1994; 1996), an addiction model utilizes the starvation as the primary force that initiates the phenomena. A serious drop in caloric intake causes an increase of endogenous opioids, which increase food consumption and slow down metabolic processes (Marrazi & Luby, 1986). The researchers argue these are response mechanisms to increase the chance of survival of the organism. In other words, the opioids lead to the ability to consume more and digest it slower, which allows it to prolong the

time interval between meals. In chronic AN, Marrazi and Luby (1986) contest that the cognitive factor of fear of gaining weight suppresses the surge in food consumption drive that endogenous opioids cause. In AA though, the intentional food deprivation forcibly reduces food consumption. Once the rats begin to eat during that restricted time, the rats can eat up to 50% of what they normally would during a 24-hour period during baseline (Gonzalez & Ernst, 2017). This suggests that the opioids are urging food consumption throughout the protocol, which is evidenced by the binging effect, observed when the forced food restriction is lifted for that hour. Marrazi and Luby (1986) do not, however, explain the role hyperactivity has in AN because there was limited research in this area at time. Now, it is shown that endogenous opioids are increased in hyperactivity (Belke, 1996) and that it has reinforcing characteristics (Pierce & Epling, 1991; 1994; Epling & Pierce, 1996; Belke, 1996; Perez-Padilla et al., 2010; Duclos et al., 2013; Giel et al., 2013). Marrazi and Luby (1986) propose that chronic activity also causes food suppression. The suppression is not in the similar competitive or interfering nature that is proposed by other researchers, but in an operant response fashion. Running is more reinforcing than eating because of the opioids. Lastly, they note that the disturbance of the estrous cycle is mediated by the endogenous opioids. These opiates affect LH and other gonadal hormones to produce amenorrhea (Marrazi & Luby, 1986). To further the involvement and possible auto-addictive hypothesis, Marrazi and Luby (1986) identify the convergent evidence provided by naloxone, an opiate antagonist. They note that it has been shown to increase weight and LH levels; both are major indicators of the pathology in AN and AA.

Aside from these potential pathways for the addictive process of AN, there are some notable physiological similarities that increased opioid levels share with AN or AA. High levels of opiates lead to a lowered body temperature (Marrazi & Luby, 1986) akin to AN (Gutierrez et

al., 2008). Water retention, constipation, lowered blood pressure, lassitude, and lowered emotional reactiveness are all proposed to be symptomatic of both pathologies (Marrazi & Luby, 1986). Thyroid functioning and HPG functioning are also affected in both AN and AA (Marrazi & Luby, 1986). These physiological changes are proposed to increase survival in a food restrictive situation by conserving energy.

Taking into consideration what Marrazi and Luby (1986) stated about AN and what new research has developed, the food deprivation causes endogenous opioids to be secreted. The opiates cause an increase in food searching and decrease in metabolic systems. The rats then increase activity due to its reinforcing properties and as a survival instinct. This causes hyperactivity and dependence on it. A convergent effect of intense exercise and a down-regulated gastrointestinal system lead to rapid weight loss as the organism must look for energy sources within. Hence, the rat develops a cachectic physique and leads to the misinterpretation as an AN effect. The rats are not self-starving themselves, but they are limited in the maximum food they can consume during that time. The rats do consume as much as they can when given the chance, which points to a more addictive behavior. The lowered weight and increased endogenous opioids cause a dysregulation of the HPG, leading to a disruption of the endogenous cycle.

Duclos et al. (2013) and Gutierrez (2013) criticize Pierce's and Epling's (1991; 1994; 1996) theory of AN as AA and point out many flaws with the theory. Gutierrez (2013) states that the reliance on psychodynamic reasons to present AN as AA makes the explanation weaker. The point Gutierrez is making is that the anorexic person "chooses" to begin dieting, exercising, and perpetuate the disorder by way of fear of overweightness. The animal is forced into the paradigm, and is reacting to survive. The anorexic person's body may be reacting to survive, but

their behavior does not change. In AA, the rats' behavior changes as soon as the food deprivation is lifted. Gutierrez (2013) notes that the resiliency of the human disorder to psychotherapeutic treatment makes the AN as AA a more difficult argument. If the cognitive distortion were to replace the forced deprivation, then treating that cognitive bias should reduce the patient's symptoms.

Duclos et al. (2013) created an experiment to test the possible behavioristic responses between an addictive behavior and a reaction to starvation on food source choice. Over multiple studies, the researchers presented animals with sucrose and saccharine food. Sucrose is energy-rich, and saccharine is an energy-less substance. They wanted to study the mediating factors of these two food sources on the development of AA and hormonal levels, mainly corticosterone. They theorized that an observed effect with saccharine would indicate a more reward-based phenomenon, while an effect of sucrose would indicate a reaction to famine (Duclos et al., 2013). They found that sucrose was the only substance that effected running and weight loss (Duclos et al., 2013). This high-energy food source reduced corticosterone in the HPA (Duclos et al., 2013), meaning the stress of the paradigm was reduced in the sucrose condition. They conclude that a reward-based response is not the driving force of the AA protocol, but the loss of a stable caloric resource causes a hyperactivity response that is self-rewarding until calories are replenished. They concluded that starvation is the only motivator in this paradigm. Running is a response to the stimulus discriminant of starvation that is positively reinforced by the effects of the endogenous opioids released.

Dwyer and Boakes (1997) also challenged Pierce's and Epling's theories by testing the response of the animals to simple changes in the feeding schedule of the AA protocol. They observed that feeding time changes, like feeding during the dark hours of the 12-hour light cycle,

could protect the animals from developing the weight loss criterion and reduced running trends (Dwyer & Boakes, 1997). Another observation for their studies was that pre-exposure or allowing for adaptation to the feeding schedule also reduced AA symptomology. If AA is to lead to either a competing hypothesis or addictive response, then the latent inhibition displayed by Duclos et al. could be the reason they observed the protective nature of pre-exposure. Starvation was not a novel stimulus to the animals. Nevertheless, running was only reduced. The animals still doubled or tripled the amount they were running during baseline.

The research shows that rats, when put on a 23-hour food deprivation schedule and given access to a running wheel, will lose weight rapidly and begin engaging in unsustainable levels of activity. The addiction hypothesis for AN states that patients will limit their intake because of the gratifying feeling they experience by not consuming calories or by engaging in activities to reduce weight. In AA, this gratifying feeling is replaced with the reinforcing properties of opiates released when engaging in exercise. This operant behavior autoshapes to higher levels of activity. Weight loss becomes rapid because of the convergent effect of hyperactivity and low caloric intake. Lastly, experimental animals consume less than control groups because of the dysregulation of the gastrointestinal system produced by the high levels of activity. It was suggested that lower consumption might be a result of ulcer development, but this was not supported because animals that did not develop ulcers still elicited the same behavior (Gutierrez, 2013). The last question that remains is what causes the phenomena in question to continue. Some researchers proposed that it is a psychodynamic drive (Routtenberg, 1987), and others theorized it is as analogous societal pressures for humans and forced deprivation on animals (Pierce & Epling, 1994). Utilizing an addiction theory, the organism is engaging in a self-reinforcing behavior. This violates the original presumption of AN as AA, because the person's

or animal's environment is continually pressuring it to respond. In an addiction theory, the organism responds to a stimulus discriminant in an autoshaping and auto-addictive fashion. The original intent of hyperactivity is for increased survivability, but it becomes extreme, unsustainable, and will lead to death.

Hypotheses

The purpose of this project is to further evaluate the AA phenomena using a different process. Much of the previous research focuses on AA utilizing a Pierce and Epling (1991; 1994) theory of AA as compared to AN. The focus of this project will be to reexamine the elicited behaviors of running. Whether it is a dependency to endogenous opioids or a reaction to a restricted feeding schedule, hyperactivity is the primary driving force of the phenomena. In a previous study, Gonzalez and Ernst (2017) point out that the anorexic pathology would lead to a stifled food consumption that would not increase. They found that rats began to increase food consumption but reached a plateau of 13 to 14 grams in that 1 hour allotted feeding period (Gonzalez & Ernst, 2017). It is argued that the rats therefore are not self-starving in any attempted means, but are physically incapable of consuming that amount of food.

In this study, activity produced by the animals will be the main concentration. With data on running being recorded every 30 minutes, the running habits of each animal will be more examinable. It is theorized an anorexic effect will cause a stable increase in activity, chiefly as an anticipatory behavior. The running should increase gradually and stabilize. If the observed running is more sporadic, then it is hypothesized that the rats may be experiencing an addictive response. The erratic nature of the running may simply be a result of energy expenditure. Running should then increase more rapidly and drop once exhaustion or inanition begins.

Secondly, half of the rats will be allowed to run during the feeding period. This will allow for studying the direct effects of the running competing with food intake levels. It is theorized that a negative correlation between running and intake would suggest an interference hypothesis. In other words, as running increases, it should cause the rat to eat less than it normally would due to suppression by increased activity. Following a dependency model, food consumption should positively correlate with running because more energy is needed to achieve those levels of hyperactivity. Endogenous opioids develop tolerance and more running, and calories, is needed to reach those levels. Furthermore, it is hypothesized that no difference between locked and unlocked wheels will be observed on daily total statistics. The subjects of both groups should engage in the same patterns of running and feeding. If the activity of the animals regulates the phenomena as hypothesized by an addiction theory, then it should increase more every test day. Conversely, the inactivity of the subjects should decrease and allow for more time to be allocated to running. Extreme exhaustion should ensue after the periods of heightened activity on the later days. As the subjects progress through the test days, effects of exhaustion and inanition should increase. However, these variables will not be measured in this project. Days in the protocol or activity sessions may be able to show some insight into these variables and their symptoms. Overall, the qualitative and quantitative review of the animals' activity is hypothesized to align more with an addiction hypothesis than the prevalent theory.

CHAPTER V

METHODS

The following methods, procedures and protocol were reviewed and approved by the Institutional of Animal Care and Use Committee (IACUC) at the University of Texas Rio Grande Valley (UTRGV; refer to Appendix B for more information regarding IACUC approval). Animals were acquired from Charles River of Kingston, New York and cared for daily by UTRGV Laboratory Animal Resources staff. No animal perished during the execution of this project.

Subjects

Thirty-two white albino female Sprague-Dawley rats were randomly assigned into four groups for testing. Female rates were selected over male rats to represent the actual disproportion of female clients' over male clients' prevalence of AN and their known susceptibility to the AA protocol. Ninety percent of clinical cases of AN are female (Williamson et al, 2004) or 10:1 ratio (APA, 2013). Each group was administered the protocol at different times due to equipment constraints. The groups' ages at the beginning of the first baseline day were 58, 83, 101, and 136 days-old. Research has indicated that an appropriate time to begin the food deprivation schedule for the rats is at 60 days-old (Perez-Padilla, Magalhaes & Pellon, 2010). The average weight for the rats was 227.38 g with a range of 164 g to 292 g. The rats were held two rats in a bin until it was time to participate in the protocol. The animals were given ad libitum food and water, and

were on a 12-hour-light/12-hour-dark light cycle. They did undergo a process of domestication by being handled on a weekly basis to ensure docility.

Apparatus and Equipment

The rats were individual cage in bins that are attached to a running wheel (Med-Associates, St. Albans, VT; Appendix C). The running wheel's axel is attached to an automatic digital wheel counter. It displayed a manual count and was connected to a computer to automatically record wheel rotations. Med-Associates' accompanying program (Med-Associates PC) on the computer was designed to output individual total wheel revolutions every 30 minutes and a daily total. This allowed for case study information based on a 30-minute interval and group data daily. The cages were placed four in line next to each other creating an "L" shaped design (Refer to Appendix C for a picture of the Lab Setup).

Rats and food were weighed using a ChefStyle digital scale that measured in whole grams. Rats were placed in a standardized box to be weighed and the weight of the box was subtracted. Food was held in individualized containers and weighed in total every day at the same time. To remain consistent, food containers were specific and respective to a rat. In other words, the rat's food was placed in the same container to be weighed and stored daily.

Rats were given a generic chow as food, and tap water was provided. The room was maintained at a constant 22.2°C and on an automatic 12-hour day/night cycle. Lights would automatically turn on at 0800 hours and turn off at 2000 hours. Access was limited to the laboratory and researchers promptly entered 5 minutes prior to completing daily measurements.

Procedure

Rat bins were randomly assigned to the different groups. Only eight rats, or four bins, were testable at a time due to equipment limitations. Rats were transferred from their normal

housing bin into the running wheel apparatus one hour prior to beginning the first 24-hour session. This allowed proper identification and equipment checks to happen before the initiation of the protocol. Rat and food weights were individually weighed prior to the rat being put into the apparatus.

The protocol gave the rats an initial five-day baseline in which food, wheel access and water were provided on an ad libitum schedule. During the ten-test days, half of the rats were given unlimited access to the wheel and water but were restricted food for 23 hours of the day. The other four rats underwent almost the exact same procedure but could not run during feeding. In other words, four rats could run during the one-hour feeding time and the other four were not allowed.

The protocols were punctually started at 1855 hours on the respective start day. During baseline, food was weighed at 1855 hours and the wheel counter was reset at 1900 hours. During test days, food was dispensed at 1755 hours, manual wheel counters were reset for unlocked wheels, and wheel were manually locked for the locked group. At 1855 hours on test days, food was recovered, weighed but not returned, the manual wheel recorder's count was recorded, and all wheels were unlocked. Likewise, the computer wheel counter was reset at 1900 hours during test days.

The rats were observed every day to ensure that their health was maintained. Five criteria were used in an integrative fashion to determine whether the animal should be removed from the protocol. Neither criteria significantly outweighed the other but were taken as piece of the whole. The first indicator was daily running count. If the rat dropped below 80% of the prior day's running total, then that would show a marked decrease in energy and a telltale sign of declining health. The second criterion was amount of food eaten. A pattern showing low amounts eaten

spanning over a couple of days would entail a dangerous low consumption to support the rat's activity. The third indicator was the rat's appearance. Many rats will develop a severe red spot on their shoulders and back, and begin to squint once they are overstressed. In addition, pale skin, as oppose to their natural pink, indicates a fainting health. The fourth criterion is the rat's motion. Once rats have reached a dangerous level of exhaustion, their gait will begin to sway and move slowly. The last indicator can only be checked once there is a concern for the rat's safety. The rat will be weighed to see if it drops below 75% of its original weight. Pierce and Epling (1994) used a 70% of original body weight disqualifier, but 75% was used in this experiment to ensure rat safety.

CHAPTER VI

RESULTS

Basic statistical analyses were completed to ensure that the observed subjects met minimal criteria for AA, as outlined by Routtenberg and Kuznesof (1967), and did not differ from group to group. Most rats (N = 20) completed the full 10 test days (T) in the deprivation protocol, but one was removed at the end of day 6, 3 on day 7, 5 on day 8, and 3 on day 9. The fourth group (58 days old) was removed on T8 to ensure the safety of the subjects from a malfunction in the facility housing the animals. As a whole, the rats lost an average of 19% of body weight from baseline day 1 (B1; M_B = 227.38 g) to T10 or discontinue (M_E = 185.19 g). Their running increased by 488% from B averages (M_B = 1282.05) to T averages (M_T = 4160.20). They also reached an asymptote of about 8 to 9 grams of food consumption during the feeding hour. From T1 to T10 food intake and running increased, but weight decreased. These trends match that of most literature regarding AA.

Weight Loss

A one-way ANOVA showed the groups significantly differed on beginning ($F(3, 28) = 48.41$, $p < 0.001$, $\eta^2 = 0.70$) and ending ($F(3, 28) = 22.15$, $p < 0.001$, $\eta^2 = 0.84$) weight amongst each other. This was expected based on the developmental effects of age on weight (refer to Table 1 and 2 for mean differences). The groups were then analyzed on an individual basis to ascertain the difference in weight between beginning and ending. Paired sample *t*-tests showed significant differences for Group 1 ($t(7) = 5.48$, $p = 0.001$, *Cohen's d* = 1.94), Group 2 ($t(7) =$

7.59, $p < 0.001$, *Cohen's d* = 2.68), Group 3 ($t(7) = 13.55$, $p < 0.001$, *Cohen's d* = 4.79), and Group 4 ($t(7) = 9.18$, $p < 0.001$, *Cohen's d* = 3.24). On average, the rats lost 19% of body weight throughout the entirety of the protocol. Group 1 lost on average 15% of body weight (M_{B1} = 218.13, M_{E1} = 184.75); Group 2 lost 18% (M_{B2} = 236.63, M_{E2} = 192.88); Group 3 lost 17% (M_{B3} = 264.25, M_{E3} = 219.25); and Group 4 lost 24% (M_{B4} = 190.50, M_{E4} = 143.88; refer to Figure 3 for visual). Overall, the rats lost a significant amount of weight that is more than the common 15% criteria for AN.

Food Consumption

The subjects ate 59% of the food at peak (M_{Tmax} = 8.31) that they were able to eat during baseline (M_B = 14.62). A one-way ANOVA showed that the groups significantly differed on baseline food consumption rates, $F(3, 156) = 6.17$, $p = 0.001$, $\eta^2 = 0.11$. Post hoc tests (Tukey HSD) revealed that only Group 3 differed from Group 1 ($p = 0.004$) and Group 4 ($p = 0.001$; refer to Table 3). While Group 3 differed from Groups 1 and 4, it was determined allowable to look at all 32 subjects together because of the small effect observed in this difference. Furthermore, an observation of the groups' data appeared to have the same trends (Figure 4 and Figure 5).

To explore further the trends of the AA protocol, food consumption was analyzed based on T Days. An ordinary least squares (OLS) regression was conducted to determine the predictability of T Days on food consumption. A significant regression equation was found, $F(1, 292) = 195.39$, $p < 0.001$, $r^2 = 0.40$. Food consumed is equal to 2.89 + 0.50 (T Day). For every T Day, food consumption increased by 0.50 grams (Figure 6). This medium to large effect on the predictability of T days on food consumption shows that the number of days in the food deprivation protocol leads to more food consumption.

Wheel Running

Generally, the rats increased their running on average by 1030% (one rat's data was not included in this base rate comparison because it produced a 64467% increase) when comparing B averages to peak running. When comparing B averages to T averages, the rats increased activity by 488% (the same rat was removed from this average because it experienced a 19203% increase). One-way ANOVA did not find an effect of different groups on baseline running, $F(3,156) = 1.25$, $p > 0.05$ (refer to Table 4). This allows for the subjects to be analyzed altogether on running behaviors (Figure 7 and Figure 8).

Similarly, subject activity was analyzed based on T Days to assess the effect of the progressive nature of the AA protocol. An OLS regression was conducted to determine the predictability of T Days on wheel running. A significant regression equation was found, $F(1, 292) = 35.01$, $p < 0.001$, $r^2 = 0.11$. Wheel running is equal to 2153.09 + 342.24 (T Day). For every T Day, wheel running increased by 342.24 revolutions (Figure 9). This small effect on the predictability of T days on wheel running shows that the number of days in the food deprivation protocol leads to more activity and, as shown earlier, feeding. This leads one to wonder what the correlation or predictability of food consumption on daily running. A significant OLS was found for the predictability of food consumption on wheel running, $F(1, 292) = 23.02$, $p < 0.001$, $r^2 = 0.07$. Wheel running is equal to 1966.92 + 365.44 (food consumed) for every gram of food intake (Figure 10).

Both food consumption and T days significantly predicted wheel running for that day. To determine which factor was more predictive, a multiple regression analysis was conducted with wheel running as the criterion and food consumption and T day as predictors. Collectively, the predictors accounted for 11% of the variance in wheel running, $F(2, 291) = 18.67$, $p < 0.001$, $r^2 =$

0.11. T days (b = 272.45, SE = 74.58, $p < 0.001$) was significantly positively associated with wheel running in the regression equation. Food consumed was, however, not significantly associated with wheel running, b = 138.96, SE = 94.02, p = 0.14. T day better predicted the amount of running that was observed versus intake amount.

Qualitative Wheel Running Analysis

With the animals' wheel activity being recorded every 30 minutes, a more qualitative approach is also possible. One can see differences in the activity patterns as T days progressed by looking at the trends of each group and all subjects altogether. The groups did not show much deviation from each other on B days (Figure 11). Altogether, the subjects recorded little to no activity from 830 hours to 1600 hours (Figure 12). During baseline, the subjects engaged in an anticipatory increase in activity two hours prior to the researchers performed daily measurement tasks. Activity markedly decreased after 1900 hours, and shortly after, peaked around 2100 hours. Their activity regresses to an approximate average of 45 revolutions per half-hour until 700 hours. The subjects' running plummets after 700 hours and hits a no response rate at 830 hours. Activity during baseline was mostly during the dark of the 12-hour light cycle. This is expected due to the animals' nocturnal nature.

Test days trends, when compared to B days, were different starting from the T1 day. On T1 (Figure 14), the established no response period (NRP) decreases from 7.5 hours to 6.5 hours and an increased peak appears at 2100 hours. Furthermore, activity did not stabilize like it did in B. In T2 (Figure 16), the NRP is reduced to 6 hours. The subjects engage in much more activity during the dark cycle and begin activity leading to the feeding period much earlier. Peak activity spikes still around the 2100 hour, but it almost double that of T1 and B. After this peak, activity gradually slopes down. T3 (Figure 18) is characterized by a large apex of revolutions from 2100

to 2200 hours. Activity, again, gradually slopes to no activity around 830 hours. The NRP lasts for 4 hours before anticipatory running begins. A second peak starts to emerge at 1700, which is just before feeding, and a noticeable valley is present after feeding. By T4 (Figure 20), the valley post-eating (VPE) is prevalent and the 2100 hours peak continues to increase. Activity more steeply decreases until 900 hours and remains at a no response rate for 1 hour. Wheel running sharply increases and peaks at 1700 hours. The VPE is still present in T5 (Figure 22), but the uniform peak at 2100 is no longer present. Running somewhat maintains at a high output, but neither stabilizes nor slowly decreases. Most noteworthy is that the NRP appears to nearly disappear. There is a low response period (LRP) from 700 to 1100 hours, but not definitive little to no activity period. The loss of the NRP is a prominent difference between the first 5 T days (T1 to T5) and the last 5 T days (T6 to T10).

Running becomes much more intense in the last 5 T days. On T6 (Figure 24), the peak of activity following the VPE is not prominent. Wheel revolutions are high (about 150 revolutions) from 2030 to 000 hours and gradually slope to 50 revolutions at 800 hours. The LRP lasts 2 hours before activity sharply increases to a peak of about 250 revolutions at 1730 hours. This peak was the highest thus far in the protocol and indicates a strong anticipatory response leading to the feeding hour. T7 (Figure 26) has the return of the peak after the VPE but is now from 2200 to 2300. Revolutions gradually reduce and maintain around 50 revolutions from 530 to 930 hours. A LRP is not overtly observable, but activity dips at 700 and 900 hours. Revolutions gradually increase to the peak at 1730 hours of 200 revolutions. On T8 (Figure 28), a bimodal distribution is present. Activity increases rapidly following the VPE and peaks around 000 hours before decreasing rapidly. No unison of LRP is visible, but two valleys at 530 and 900 are present. Revolutions moderately increase to a second apex of about 220 revolutions at 1730

hours. T9 (Figure 30) is characterized by sustained high activity during the dark cycle and multiple peaks. Five peaks appear at 1900, 2200, 300, 1130, and 1730 hours. Valley are present at 1930 (the VPE), 830 to 1000, and 1330 hours. High running is maintained from 2100 to 400 hours before entering a LRP from 500 to 800 hours. It then dips into a NRP from 830 to 1000. Running peaks, drops, and rapidly peaks before the eating hour. The last day of protocol (T10; Figure 32) has the most variable running pattern of all the days and the highest peaks of all the T days. Hourly peaks followed by hourly valleys riddle this day. The VPE has somewhat vanished from the trend. A LRP is distinguishable from 630 to 830 hours, but the running moderately increases to about 270 revolutions prior to eating. The last few days have a high rate of running most of the day follow by valleys scattered through the day.

By comparison, the B data was much lower than the T days (Figure 37). When comparing the first 5 and last 5 test days, the running became more intense and the NRP converted into a LRP. No definitive NRP was present in any of the T days past T4. Through most of the days, the VPE was present and always was at 1930 hours. Anticipatory running increased daily leading up to the feeding hour. A second differing characteristic of the first 5 and last 5 is that T6 to T10 have a higher peak of activity prior to eating than the apex around 2100 hours. The trend through most of the protocol is the subjects moderately increases activity starting around 1000 hours, peak just before eating, enter a LRP immediately after the eating hour (referred to as the VPE), and increasing rapidly to a peak around 2100 hours. Revolutions after these peaks varies from day to day, and becomes more intense.

Activity and Inactivity. The analysis of running every 30 minutes produced a simple dichotomous variable of active (A) and inactive (I) sessions. From this frequency of A and I sessions, one can produce rates of activity. In other words, the previous qualitative look at the

30-minute intervals only shows trends by time. However, one can also infer about the amount of increase in running by looking at the rate of activity. During B, the subjects were active an average of 45% of the session or 21.70 (SD_B = 7.04) of the 48 sessions. A increased almost every day and ended at 63% or 30.15 (SD_{T10} = 4.42) on T10 (Figure 38). An OLS regression was conducted to test the predictability of days on A and to further analyze the trend. A significant regression equation was found, $F(1, 292) = 63.62$, $p < 0.001$, $r^2 = 0.18$. A sessions is equal to 22.67 + 0.98 (T Day). For every T Day, A sessions increased by 0.98 sessions (Figure 39). This small effect on the predictability of T days on A sessions shows that the number of days in the food deprivation protocol leads to more activity. Inversely, I was decreasing. Every day I was reduced by 0.98 sessions. This matches the loss of the NRP during the last 5 T days.

Running Versus Feeding

One of the main relationships that this study focused on was allowing for running to interfere with eating. Half of the subjects could run or eat during the feeding hour (referred to as the "unlocked group"; UL) while the others were not permitted (referred to as the "locked group"; L). Wheel rotations during the feeding hour was significantly positively correlated with food consumption, $r(145) = 0.27$, $p < .001$, $r^2 = 0.07$. As running during the feeding hour increased, food consumption also increased for the UL group.

An OLS regression was conducted to assess the predictability of food consumption on wheel running during the feeding hour for the UL group, because of the significant correlation found. In essence, the regression would tell more about this relationship than the correlation. Food consumption did significantly predict wheel running, $F(1, 143) = 11.44$, $p = 0.001$, $r^2 = 0.07$. Wheel running is equal to 74.95 + 16.09 (food consumption); each gram of food consumed increased wheel running by 16.09 revolutions (Figure 40). A multiple regression analysis was

conducted to assess whether food intake or T day predicted wheel rotations during the feeding hour. A significant regression equation was found for these two predictors which accounted for 10% of the variance, $F(2, 142) = 7.63$, $p = 0.001$, $r^2 = 0.10$. Food ($b = 23.96$, $SE = 6.27$, $p < 0.001$) was positively significantly associated with wheel revolutions during the feeding hour, but T days ($b = -9.71$, $SE = 5.10$, $p = 0.05$) was not. This was the first regression in which T days did not predict a dependent variable. Food was the better predictor of wheel rotations during the allotted one hour of feeding.

Locked Versus Unlocked

Another point of emphasis involved in this research design was to see the overarching differences among the "unlocked" (UL) and "locked" (L) groups. The L's were not allowed to run during the feeding time. As indicated in the previous section, the ability to run while eating led to higher rates of wheel activity as food consumption increased. In a direct comparison, neither group lost more weight percentage-wise ($t(30) = -0.95$, $p = 0.35$) nor was one removed from the protocol at an earlier time ($t(30) = 0.58$, $p = 0.57$). On baseline values, L and UL groups did not vary on food consumption rates ($t(158) = 0.46$, $p = 0.65$; Figure 41), but did significantly differ on running rates ($t(157) = -4.39$, $p < 0.001$, *Cohen's d* = 0.70; Figure 42). UL subjects ($M_B = 1521.88$, $SD_B = 855.10$) had the propensity to run more during B days than L subjects ($M_B = 981.63$, $SD_B = 690.14$). Further analysis showed that 4 subjects from the L group engaged in less than 250 revolutions per day. These subjects' data was still used for further analysis.

A 2 (Lock; L and U) X 10 (Days) repeated measures factorial ANOVA was conducted with food consumption as the dependent variable. A significant main effect was found for Days ($F(9, 63) = 28.55$, $p < 0.001$, *partial* $\eta^2 = 0.15$), but no effect was found for Lock ($F(1, 7) = 1.21$, $p = 0.31$) or the interaction of Lock X Days ($F(9, 63) = 0.58$, $p = 0.81$). Similarly, another 2

(Lock; L and U) X 10 (Days) repeated measures factorial ANOVA was completed with wheel running as the dependent variable. Again, a significant main effect was found for Days ($F(9, 63) = 18.34$, $p < 0.001$, *partial* $\eta^2 = 0.72$), but no effect was found for Lock ($F(1, 7) = 3.33$, $p = 0.11$) or the interaction of Lock X Days ($F(9, 63) = 0.92$, $p = 0.51$). This was expected based on the significant regression equations found earlier. There was no difference between the L and UL groups in terms of running or food intake throughout the T days (Figures 43 and 44). The ability to run during the feeding hour had no effect on daily food consumption or activity rates.

Extrapolated Data

Many animals ($N = 12$) were not able to finish all 10 days of the test protocol and were removed. All of Group 4 was removed for safety on the 8th day. This leaves the observed difference between UL and L rats limited. Only nine subjects from the UL condition completed all 10 T days, while eleven subjects form the L condition completed them (Figure 45). To better analyze the true effect of L versus UL conditions, data for food consumption and wheel was extrapolated. Individualized OLS regressions were used, when significant at the $p < 0.05$ level, to extrapolate for the missing data (Table 5; Table 7). If a nonsignificant OLS regression was not found, then the group's OLS regression was used to predict the data (Table 6, Table 8). Individual data graphs are given for the subjects with missing data to show the fit of the extrapolated data to the rat's trend (Figures 46 to 69) The above statistics were redone using this extrapolated data to reanalyze the effect a locked wheel had on the protocol.

Two - 2 (Lock; U and L) x 10 (Days) repeated measures factorial ANOVA's were done to assess for an effect on wheel running and food consumption with the new extrapolated data. For food intake, a significant main effect was found for Days ($F(9, 135) = 32.25$, $p < 0.001$, *partial* $\eta^2 = 0.68$). There was no effect found for Lock ($F(1, 15) = 0.82$, $p = 0.38$) or the

interaction effect of Lock X Days ($F(9, 135) = 0.49$, $p = 0.88$) for food intake. As for wheel running, a significant main effect was found for Lock ($F(1, 15) = 4.85$, $p = 0.04$, *partial* $\eta^2 = 0.24$) and for Days ($F(9, 135) = 20.29$, $p < 0.001$, *partial* $\eta^2 = 0.58$). Subjects in the UL group ($M_{UL} = 4788.24$, $SE_{UL} = 518.64$) significantly ran more than the L group ($M_L = 3716.66$, $SE_L = 606.42$). No interaction effect was found for Lock X Days ($F(9, 135) = 1.82$, $p < 0.07$) for wheel running. Once again, Days was the only factor that influenced food consumption and wheel running, but the Lock did affect wheel running (Figures 70 and 71). This may be due to the natural propensity to run more by UL rats prior to beginning the test days, as discussed earlier, or the slight increase in wheel running caused by the extra hour of running.

To control for the higher baseline rates of UL rats, a 2 (Lock; U and L) x 10 (Days) repeated measures factorial ANCOVA was conducted to observe the main effect of a locked wheel or test day on the rats while controlling for baseline rates. In other words, is there a significant difference between L and UL groups if we control for their propensity to run during baseline? A significant main effect was still found for Days ($F(9, 261) = 4.25$, $p < 0.001$, *partial* $\eta^2 = 0.13$), and an interaction effect was found for Days X Baseline ($F(9, 261) = 2.16$, $p = 0.03$, *partial* $\eta^2 = 0.07$). However, no main effect was found for Lock ($F(1, 29) = 0.04$, $p = 0.85$) or the interaction of Day X Lock ($F(9, 261) = 0.88$, $p = 0.54$). Once the lower initial running rates for L subjects was controlled for, the main effect between L and UL groups ceased to be significant.

CHAPTER VII

DISCUSSION

The main intent of this project was to reanalyze the AA protocol without focusing on the weight loss or food consumption that many researchers have done before. Its main goal was to focus on the overt behavior of activity from another perspective that was not dominated by the prominent AN as AA theory. The subjects involved did not differ from previous research and showed that the behaviors are not as Pierce and Epling (1991; 1994; 1996) initially proposed them to be. Weight was significantly decreased; running was drastically increased; and food intake reached an average asymptote of 9 grams. The AA phenomena was replicated in these subjects, but a small caveat was added to the protocol.

Allowing half of the subjects to run during the feeding hour permitted the direct effect of an unlocked wheel to be observed. No effect was found, however, from having the wheels unlocked. This meant that the animal's ability to run while feeding had no effect on the development of the AA phenomena. Contrary to Pierce and Epling's theory, the running did not interfere with the ability of the subject to feed. Moreover, the amount of food consumed could predict the amount of running that happened that day. In other words, the results of this project support the logical idea that food intake should govern the amount of wheel running. The idea of food consumption suppression through the influx of β-endorphins was not supported; however, the proposition of gastrointestinal suppression and increased appetite might still be supported. No control groups were used in this project, but review of the literature shows an average

asymptote of 13 grams for control animals in a 23-hour food deprivation protocol over 10 days (Routtenberg & Kuznesof, 1967). The subjects in this project still consumed about 4 grams less than controls within that hour. The increase in endogenous opioids could be causing the gastrointestinal process to slow down, including limiting stomach expandability or gastric capacity. Limited research has been completed on gastric capacity in relation to exercise or dieting. A study by Geliebter et al. (1996) found that participants had a lower gastric capacity after completing a strict dieting regimen. The effect exercise or a restricted calorie regimen has on the stomach's capacity is still debatable, but it may be the reason for the difference in consumption rates for experimental and control subjects.

A major novelty of this project was to review the activity of the subjects every 30 minutes throughout the entirety of the protocol. This quantitative data was reviewed qualitatively to reveal some surprising trends over the 10 T days and 5 B days. During baseline, the rats mostly ran during the night and were inactive from 800 to 1600 hours. They only began increasing activity a couple of hours before the researchers entered the room to complete daily measurement tasks. All the groups consistently showed this trend during baseline. As soon as the protocol began, this pattern was disrupted and morphed into bimodal running sessions with a consistent VPE. Running was not just performed at night, but in an intense fashion prior to the feeding period. This anticipatory response indicates the level of importance the feeding period became to the animal. The response rate almost matched that of scalloping behavior, but the initial respond interval during the dark does not.

The appearance of the VPE is something that was not discussed in previous literature. The animals enter a 30-minute interval immediately after the feeding hour in which activity drops to one of the lowest in the 24-hour session. It could be characterized to say that the feeding

is interfering with the running or their hunger was temporarily satiated. Another possibility is that the binging behavior and slowed metabolic system that interferes with the running. Temporary satiation of the rat's hunger is the more likely result. On a more physiological plane, the corticosterone levels of the rat could reduce significantly after feeding causing a reduction in food seeking behavior. Duclos et al. (2009) experiments concluded the significance of corticosterone in the development of AA and noted the positive correlation between corticosterone levels and wheel activity. It is suggested that the food deprivation causes corticosterone levels to raise; these level lead to increased activity (Duclos et al., 2009). During the VPE, corticosterone could subside as the hunger of the rat is temporarily satiated. After the hour, the gastrointestinal system processes the consumption and reestablishes the stress of food deprivation. This could account for the peak of activity following immediately after the VPE. Corticosterone decreases post feeding-hour, increases during the VPE, peaks before the 2100 hours, and returns to a stable level by 2400 hours. This project did not include an intravenous or an internal aspect, and corticosterone levels were not measured. Based on Duclos et al.'s (2009) findings, it is suggested corticosterone levels may explain the appearance of the VPE. Further analysis, such as video evidence, can help to answer the actual behaviors the animals engaged in or the effect of corticosterone to wheel activity during the VPE.

The rats progressively limited their inactivity during the light cycle. This indicated both a loss in normal sleep patterns and a significant response to run. Anorexia nervosa is not associated with a disturbance in sleep (Pieters et al., 2004) or sleep patterns (Latzer, Tzischinsky & Epstein, 2001), but substance abuse is (Mahfoud et al., 2009; Hasler et al., 2012). The loss of sleep in addictions is a convergent effect of many causes. Most of these disturbances are due to the stimulating nature of the substance, like amphetamines, or the withdrawal (Mahfoud et al.,

2009). Endogenous opioids, part of the opiate class, are natural pain relievers, highly addictive, and released during exercise. In studies concerning the use of opioid medication on sleep, researchers found that this medication exacerbated the sleep problems (Robertson et al., 2016). The surge in endorphins could not only reinforce the activity, but it may disturb the animal's sleep/wake cycle or ability to rest. Mahfoud et al. (2009) point out that in comparisons of rat wheel activity and alcohol use, the sleep cycles were similarly disturbed. The rewarding nature of wheel running for rats can parallel that of a substance abuse and cause similar sleep-loss behavior. This could further explain the fast development of the phenomena and the loss of the NRP and LRP. Starvation and intense activity should cause exhaustion because there is rapid calorie expenditure without the resource to replenish it. A disruption of the sleep cycle can add to this exhaustion. This could help explain the rapid progression to death for these animals on an AA protocol. Lastly, sleep is modulated by the suprachiasmatic nucleus which is in the hypothalamus. With much research pointing to disruptions in the HPA and HPG, it is also possible this hypothalamus-based process is also affected. More research into AA's disruption of the sleep cycle is needed to confirm this effect, because this project only focused on the daily loss of inactivity sessions.

For the most part, the groups did not differ across the various dependent variables except weight. Age, sex, and developmental stage of the rat contributes to the weight of the rat. Considering a female rat reaches sexual maturity at approximately 40 days old and adulthood at 60 days old (Sengupta, 2013), the age difference between the groups influenced this statistic. Nevertheless, each group lost an equivalent percentage of weight. Food consumption appeared similar across the subjects and an average asymptote at 8 grams/hour was established. The only main effect found was the T days. Running and food consumption progressively increased as the

days continued, which coincides with previous research. The longer the animals are in the food deprivation, the more intense their activity becomes and the subjects reach the max amount of food consumable in one hour.

The increase of activity is not sustainable and, as Routtenberg and Kuznesof (1967) point out, the animals will parish. This is explainable by the limited amount of nourishment the animals are able to consume and the level of physical activity they will engage in. Physical activity is limited by the amount of caloric intake. As the external caloric intake becomes insufficient to sustain the animal's physical activity, it begins to burn stored fats. Eventually there will come a point where the animal's natural internal reserve of sustenance will be exhausted. This is the dangerous part of the protocol in which inanition and death are possible. At this point exhaustion should stop activity, but Routtenberg's and Kuznesof's (1967) data show that the animal will continue to run even when this threshold is theorized to occur. While the food deprivation explains the lack of nutrition, it is an insufficient to reason for the continued exertion. If survival forces, are the initial instigating factor for the rat's running, then should these forces reappear to assist in survival when the rat passes or nears the calorie-loss threshold? This project did not allow the animals to reach this stage and were removed from the protocol when it appeared that the subject was nearing this threshold. Thus, this project is unable to analyze this point of the phenomena.

Both anorexia nervosa and the opioid substance abuse DSM-5 criteria have a criterion of persistent behaviors even if it the behaviors cause or maintain distress. This qualifier in many situations leads to the death of the patient for both disorders. The main difference between a substance abuse and anorexia nervosa is that the cause of death in anorexia nervosa is calorie restriction. In substance abuse, it is usually due to an overdose or accident while on the mood

altering substance. This more parallels the overexertion observed before a subject parishes in AA. In essence, the animal overdoses on activity and pushes its body past the threshold of exhausted calories. The body is unable to compensate or react to the low level of calorie and the animal parishes.

Recent literature has begun noting a cluster of symptoms called exercise dependence. Researchers observed this pathology in bodybuilders and marathon runners, and found no significant gender bias (Smith, Wright & Winrow, 2010). Intense level of physical activity, amount of time dedicated to such activities, persistence of behavior even if physically hurtful (e.g. running on a sprained ankle), feeling obligated to exercise, and experiencing withdrawal symptoms after stopping are indicative of an exercise dependence (Smith, Wright & Winrow, 2010). There is some semblance to this pathology and the effects seen in AA. The literature for both phenomena implicates endogenous opioids, but no reported case of a person exerting him- or herself to death was found. One pattern researchers noted is that early phases of exercise dependence is initiated by the person's desire to obtain a better physique (Smith, Wright & Winrow, 2010). By the latter stages, the person no longer reports wanting that physique as his or her primary goal, but reports wanting to maintain his or her physical ability as the main goal (Smith, Wright & Winrow, 2010). More research into exercise dependence is necessary to properly compare the two phenomena.

CHAPTER VIII

CONCLUSION

Gutierrez (2013) argues the fact that this phenomenon is activity-based not anorexic-based. By focusing only on the running response of the subjects in the AA protocol, some trends were noted. The rat's running did not only increase, it increased throughout the progression of the protocol. This lead to a disruption of the sleep cycle of the animals and an anticipatory response to appear. Allowing the animals to run during the feeding hour did not result in a significant effect. The animals still responded to the environmental situation in the same manner. Wheel running, as described by Marrazi and Luby (1987), can explain most of the phenomena in conjuncture with the food deprivation. Weight loss is due to the convergent effect of intense activity and restricted calorie intake. The running is initiated by the starvation but is autoshaped by the activity's rewarding factors. The lowered eating in the feeding hour is because of the slowed down gastrointestinal process. Control rats are not allowed to run during AA so they do not experience the surge in β-endorphins that may account for the limited feeding capacity. This projects results support the theory that the protocol is activity-based, and the other symptoms noted by other measures are part of the side effects of the extreme activity.

Future research in the AA protocol should focus on finding more definitive evidence of the actual neurotransmitters involved in AA. Many theories are proposed, but no study can unequivocally state what neurological processes are involved. Furthermore, the proposed pathways of the HPA and HPG are not definitive either. The hormones involved in these axes

affect numerous systems and organs. Research points to β-endorphins, but dopamine has also been suggested. Corticosterone can also be the leading hormone involved, but no one is sure. Use of this phenomenon to develop pharmaceutical interventions is futile until the underlying processes are identified, unless the intervention only aims to reduce activity levels.

The term treximomania (Gonzalez & Ernst, 2017) has been proposed to better label the AA protocol. This term is proposed because it means “running addiction”. Botello et al. (2018) suggest that the increased activity in the AA protocol is more addictive than anorexic. The subjects’ running increased rapidly and did not show a plateau until the threshold of inanition began. The ability to run themselves to death is not an anorexic affect, but an addictive one. The natural rewarding nature of wheel running for rats creates a cycle that autoshapes ever-increasing levels of activity. Other convergent evidence, such as sleep-loss, also support the proposed addictive hypothesis for AA.

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APPENDIX A

APPENDIX A

DSM-5 Anorexia Nervosa Diagnostic Criteria

APA (2013) lists the following as Diagnostic Criteria for Anorexia Nervosa:

A. Restriction of energy intake relative to requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health. *Significantly low weight* is defined as a weight that is less than minimally normal or, for children and adolescents, less than that minimally expected.
B. Intense fear of gaining weight or of becoming fat, or persistent behavior that interferes with weight gain, even though at a significantly low weight.
C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight.

Specify whether:

Restricting type
Binge-eating/purging type

Specify if:

In partial remission
In full remission

Specify current severity:

Mild: BMI $\geq$ 17 kg/m^2
Moderate: BMI 16 – 16.99 kg/m^2
Severe: BMI 15 – 15.99 kg/m^2
Extreme: BMI < 15 kg/m^2

APPENDIX B

APPENDIX B

IACUC APPROVAL

The following are the reference numbers for IACUC approval and their respective effective dates. This project was completed in conjuncture with an outreach camp for local middles school students.

AUP 2017-001 – Inspiring STEM Educational Interest in Under-Resourced Middle School Children: A Proof of Concept Program

Effective: 1 February 2017 to 15 September 2017

AUP 2017-002 – Inspiring STEM Educational Interest in Under-Resourced Middle School Children: A Proof of Concept Program

Effective: 15 September 2017 to 21 March 2018

APPENDIX C

APPENDIX C

APPARATUS AND LAB LAYOUT

Figure 1 – Med Associates Activity Wheel – This figure shows the activity wheel used in this project. Image was retrieved from http://www.med-associates.com/product/activity-wheel-with-plastic-home-cage-for-rat/

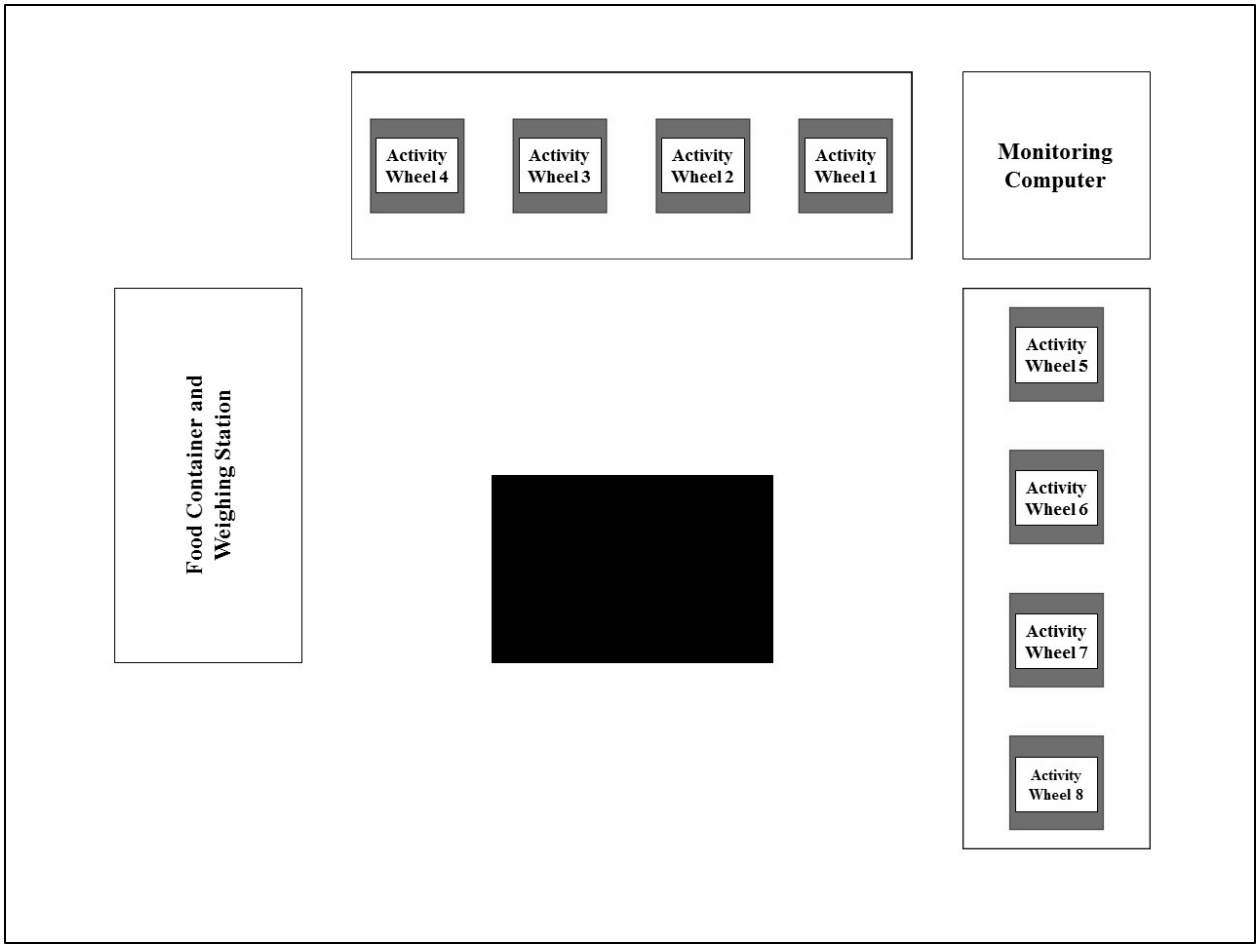


Figure 2 – Sketch of Laboratory – This figure shows the layout of the laboratory the project was conducted in.

APPENDIX D

APPENDIX D

TABLES

Table 1 – Mean Differences Between Groups on Beginning Weight

Table 1 – Mean Differences Between Groups on Beginning Weight

	M	*SD*	Group 1	Group 2	Group 3	Group 4
Group 1	218.12	7.77	-	-18.5[a]	-46.12[c]	27.62[b]
Group 2	236.62	12.12	18.5[a]	-	-27.62[b]	46.12[c]
Group 3	264.25	15.20	46.12[c]	27.62[b]	-	73.75[c]
Group 4	190.50	14.09	-27.62[b]	-46.12[c]	-73.75[c]	-

One-way ANOVA produced a significant difference among the groups on Ending Weight, $F(3, 28) = 48.41$, $p < .001$. Tukey's HSD post hoc test was used to assess significance between groups. All units are in grams.

[a] – Indicates significance at the $p < .05$.

[b] – Indicates significance at the $p < .01$.

[c] – Indicates significance at the $p < .001$.

Table 2 – Mean Differences Between Groups on Ending Weight

Table 2 – Mean Differences Between Groups on Ending Weight

	M	*SD*	Group 1	Group 2	Group 3	Group 4
Group 1	184.75	18.12	-	-8.12	-34.50[b]	40.87[b]
Group 2	192.87	25.77	8.12	-	-26.37[a]	49.00[c]
Group 3	219.25	15.61	34.50[b]	26.37[a]	-	73.37[c]
Group 4	143.87	13.15	-40.87[b]	-49.00[c]	-73.37[c]	-

One-way ANOVA produced a significant difference among the groups on Ending Weight, $F(3, 28) = 22.15$, $p < .001$. Tukey's HSD post hoc test was used to assess significance between groups. All units are in grams.

[a] – Indicates significance at the $p < .05$.

[b] – Indicates significance at the $p < .01$.

[c] – Indicates significance at the $p < .001$.

Table 3 – Mean Differences Between Groups on Baseline Consumption

Table 3 – Mean Differences Between Groups on Baseline Consumption

	M	*SD*	Group 1	Group 2	Group 3	Group 4
Group 1	15.45	3.64	-	0.92	2.77[a]	-0.40
Group 2	14.52	3.54	-0.92	-	1.85	-1.32
Group 3	12.67	3.94	-2.77[a]	-1.85	-	-3.17[a]
Group 4	15.85	3.22	0.40	1.32	3.17[a]	-

One-way ANOVA produced a significant difference among the groups on Baseline Consumption, $F(3, 156) = 6.17$, $p < .01$. Tukey's HSD post hoc test was used to assess significance between groups. All units are in grams.

[a] – Indicates significance at the $p < .01$.

Table 4 – Mean Differences Between Groups on Baseline Running

Table 4 – Mean Differences Between Groups on Baseline Running

	M	*SD*	Group 1	Group 2	Group 3	Group 4
Group 1	1252.44	805.35	-	24.34	185.88	-169.44
Group 2	1228.09	743.65	-24.34	-	161.54	-193.79
Group 3	1066.56	469.10	-185.88	-161.54	-	-355.33
Group 4	1421.88	1130.38	169.44	193.79	355.33	-
One-way ANOVA did not produced a significant difference among the groups on Baseline Consumption, $F(3, 156) = 1.25$, $p > .05$. All units are in grams.						

Table 5 – Individual Rat OLS Regressions for Extrapolating Food Consumption

Table 5 – Individual Rat OLS Regressions for Extrapolating Food Consumption

Rat	*df*	*F*	r^2	*SE*	Predicted Values Day	Predicted Values Value
Rat 5	1, 7	158.15[c]	0.96	0.07	10	9.52
Rat 13	1, 6	6.07[a]	0.50	0.13	10	6.21

Individual OLS regressions were used to predict the extrapolated values above. Each rat's significant regression statistics are given including standard error for this estimate.

[a] – Indicates significance at the $p < .05$.

[c] – Indicates significance at the $p < .001$.

Table 6 – Group OLS Regression for Extrapolating Food Consumption

Table 6 – Group OLS Regression for Extrapolating Food Consumption

	Predicted Value	
Rat	Day	Value
Rat 7	9	8.73
	10	9.39
Rat 17	10	6.76
Rat 25	9	6.74
	10	7.09
Rat 26	7	6.06
	8	6.40
	9	6.74
	10	7.09
Rat 27	9	6.74
	10	7.09
Rat 28	8	6.40
	9	6.74
	10	7.09
Rat 29	8	6.40
	9	6.74
	10	7.09
Rat 30	8	6.40
	9	6.74
	10	7.09
Rat 31	9	6.74
	10	7.09
Rat 32	9	6.74
	10	7.09

Group OLS regressions were used to extrapolate the missing values for the indicated subjects. For Rat 7 the regression for Group 1 ($SE_1 = 0.07$) was used, $F(1, 75) = 91.64$, $p < 0.001$, $r^2 = 0.55$. For Rat 17, the regression for Group 3 ($SE_3 = 0.05$) was used, $F(1, 77) = 58.04$, $p < 0.001$, $r^2 = 0.43$. For rats Rat 25 through Rat 32, the regression for Group 4 ($SE_4 = 0.12$) was used, $F(1, 57) = 8.86$, $p = 0.004$, $r^2 = 0.13$.

Table 7 – Individual Rat OLS Regressions for Extrapolating Wheel Running

Table 7 – Individual Rat OLS Regressions for Extrapolating Wheel Running

					Predicted Values	
Rat	*df*	*F*	r^2	*SE*	Day	Value
Rat 5	1, 7	12.56[b]	0.64	97.61	10	4168.41
Rat 7	1, 6	6.36[a]	0.52	367.98	9	10915.76
					10	11843.92
Rat 13	1, 4	31.64[b]	0.89	232.93	10	2806.55
Rat 17	1, 7	21.12[b]	0.75	159.26	10	7509.24
Rat 25	1, 6	35.70[b]	0.86	126.12	9	5371.40
					10	6124.94
Rat 29	1, 5	11.59[a]	0.70	308.04	8	9161.89
					9	10210.66
					10	11259.43
Rat 31	1, 6	6.78[a]	0.53	129.97	9	6709.43
					10	7047.75

Individual OLS regressions were used to predict the extrapolated values above. Each rat's significant regression statistics are given including standard error for this estimate.
[a] – Indicates significance at the $p < .05$.
[b] – Indicates significance at the $p < .01$.

Table 8 – Group OLS Regression for Extrapolating Wheel Running

Table 8 – Group OLS Regression for Extrapolating Wheel Running

	Predicted Value	
Rat	Day	Value
Rat 26	7	7161.23
	8	7667.86
	9	8174.48
	10	8681.10
Rat 27	9	8174.48
	10	8681.10
Rat 28	8	7667.86
	9	8174.48
	10	8681.10
Rat 30	8	7667.86
	9	8174.48
	10	8681.10
Rat 32	9	8174.48
	10	8681.10

A group OLS regression was used to extrapolate the missing values for the indicated subjects, $F(1, 57) = 7.26$, $p = 0.009$, $r^2 = 0.11$. The standard error for these estimations is 188.00 revolutions.

APPENDIX E

APPENDIX E

FIGURES

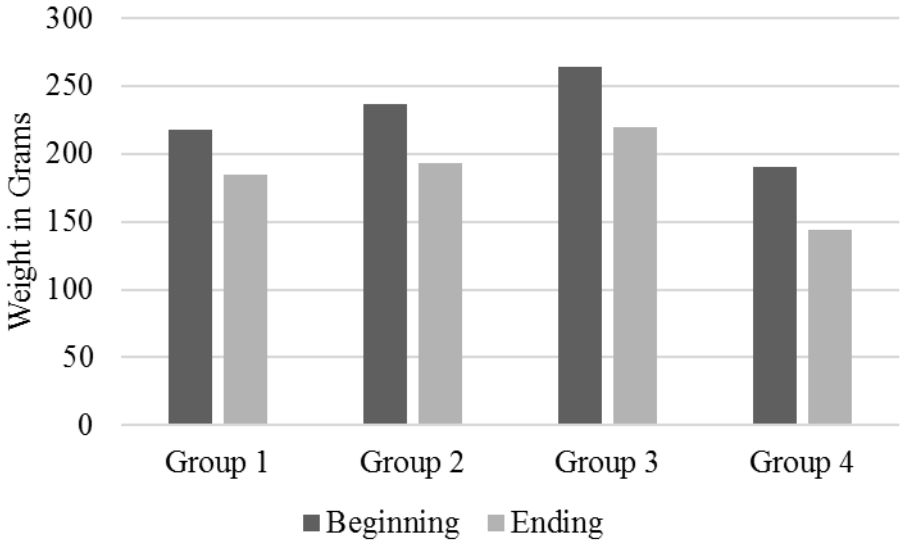


Figure 3 – Beginning and Ending Means by Group – This figure shows the mean beginning and ending weights for each group. All groups weight changes were significantly different at the $p < .001$, except Group 1 which was at $p < .01$.

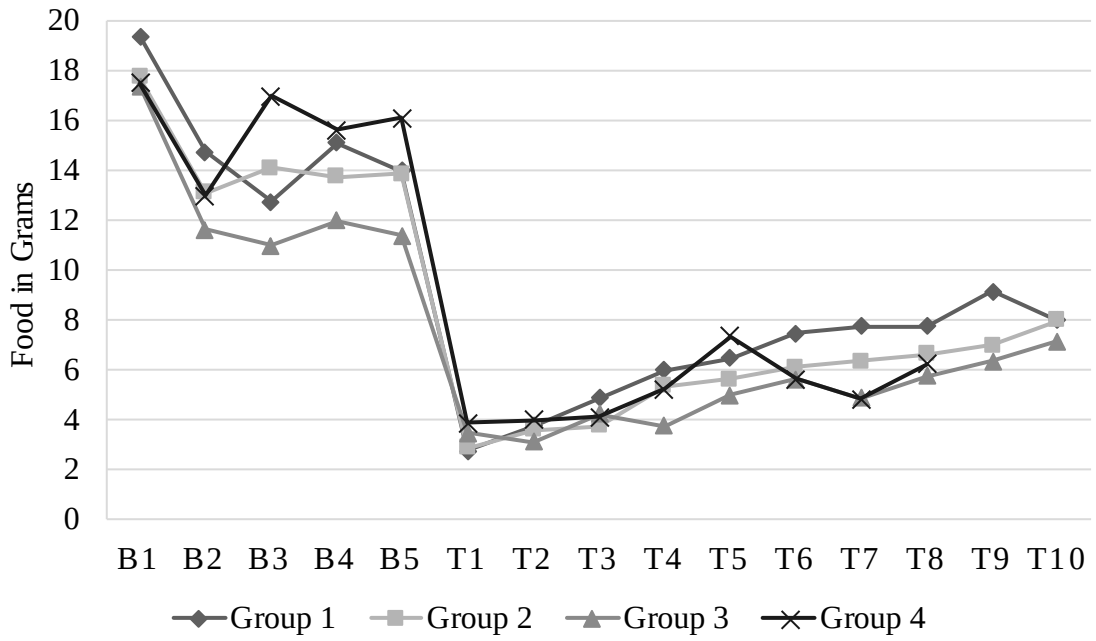


Figure 4 – Average Food Consumption by Group per Day – This figure shows the daily average of food consumption per group.

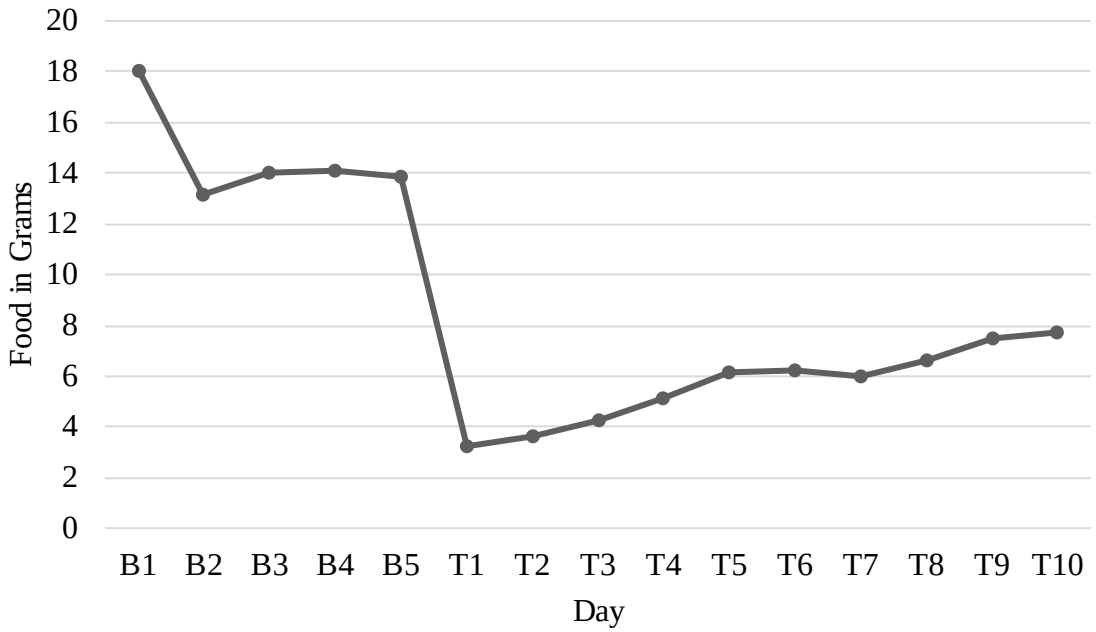


Figure 5 – Average Food Consumption Per Day – This figure displays the average food consumption for all subjects (*N* = 32).

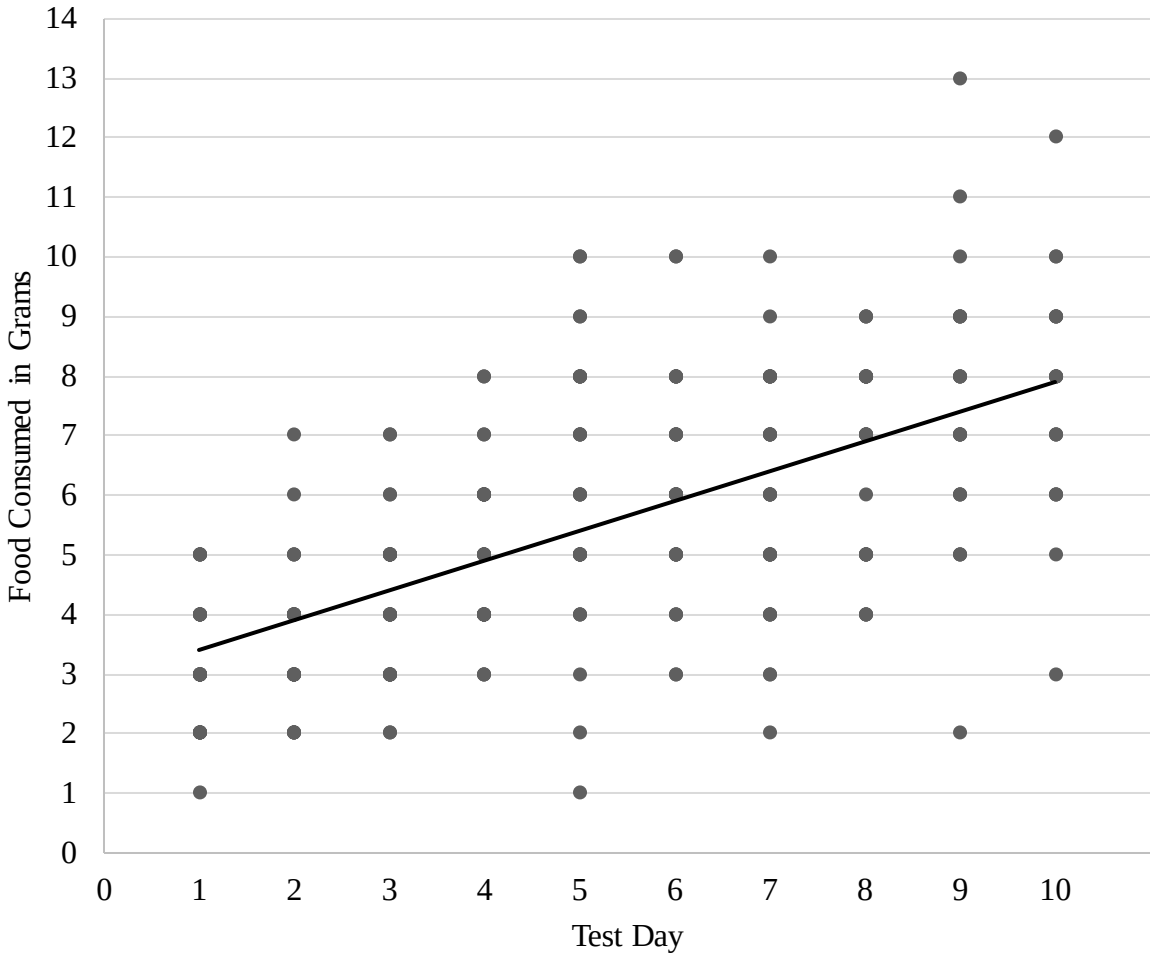


Figure 6 – Scatterplot of Food Consumption by Test Day – This figure shows the amount of food consumed per T Day for all subjects (N = 32). It also includes the significant regression line that predicted food consumption based on T Day, $F(1, 292) = 195.39$, $p < 0.001$, $r^2 = 0.40$. Food consumed is equal to 2.89 + 0.50 (T Day). For every T Day, food consumption increased by 0.50 grams.

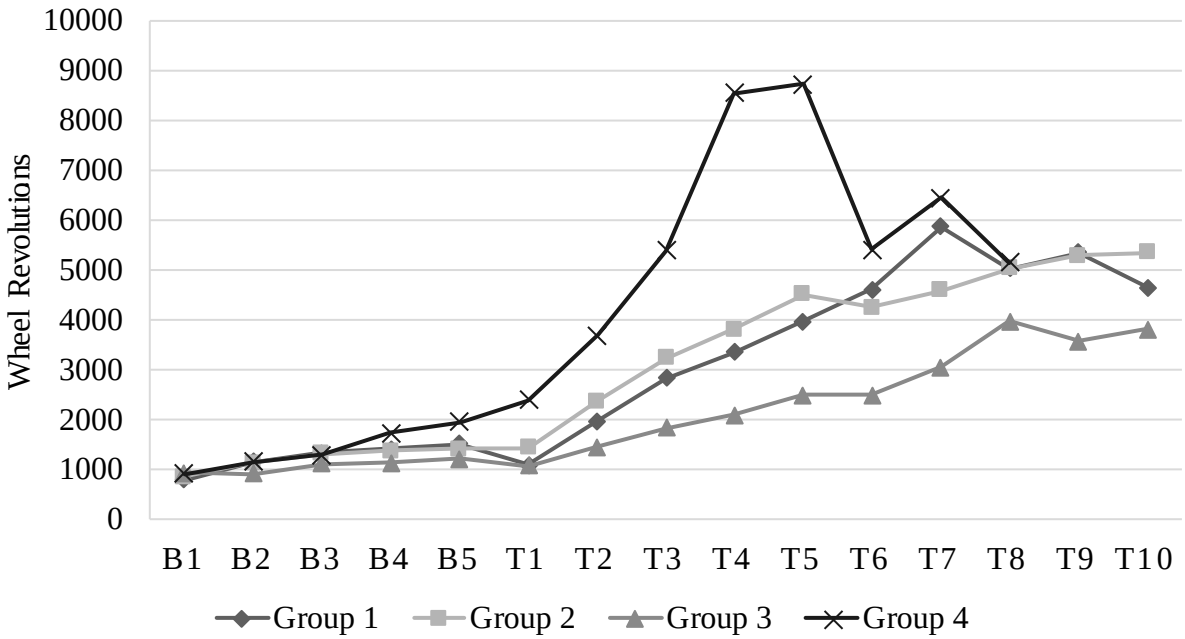


Figure 7 – Group Average Running by Day – This figure displays the average wheel revolutions completed by each group by day.

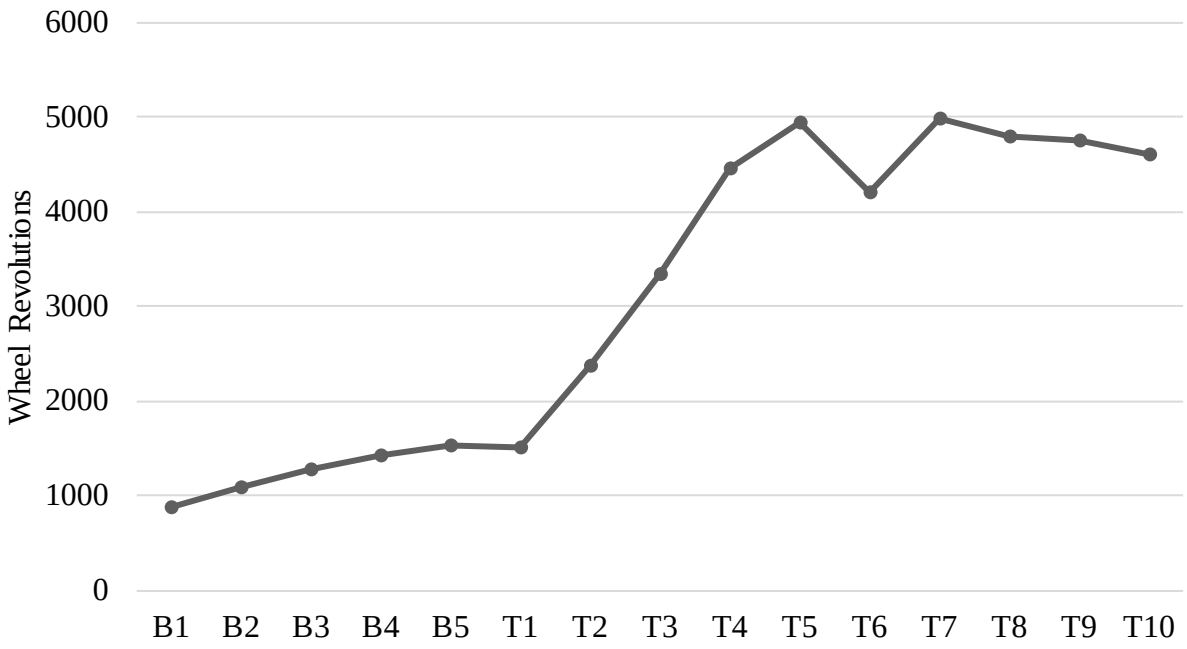


Figure 8 - Average Running Per Day – This figure displays the average wheel revolutions for all subjects (*N* =32).

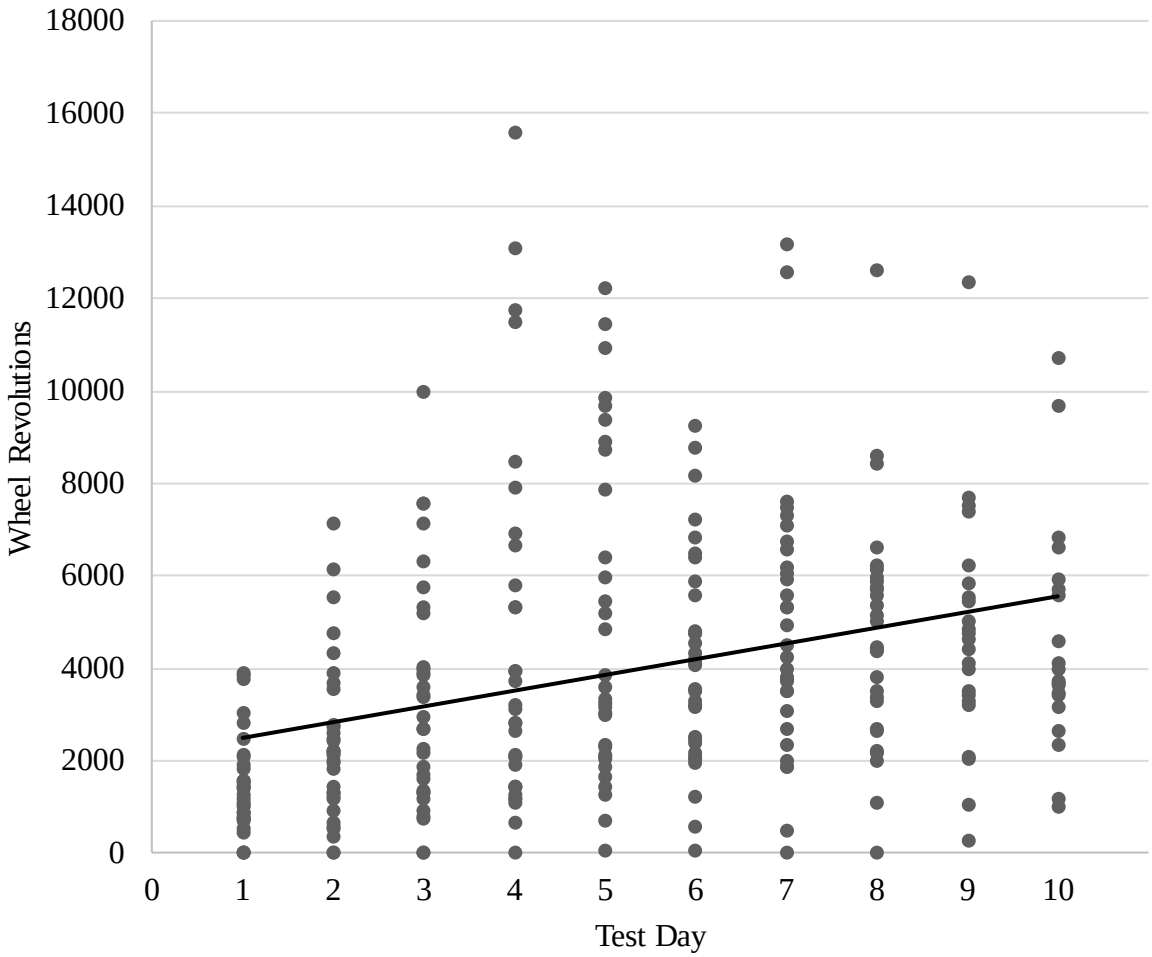


Figure 9 – Scatterplot for Daily Wheel Running per T Day – This figure shows the amount of wheel running per T Day for all subjects (N = 32). It also includes the significant regression line that predicted wheel running based on T Day, $F(1, 292) = 35.01$, $p < 0.001$, $r^2 = 0.11$. Wheel running is equal to 2153.09 + 342.24 (T Day). For every T Day, wheel running increased by 342.24 revolutions.

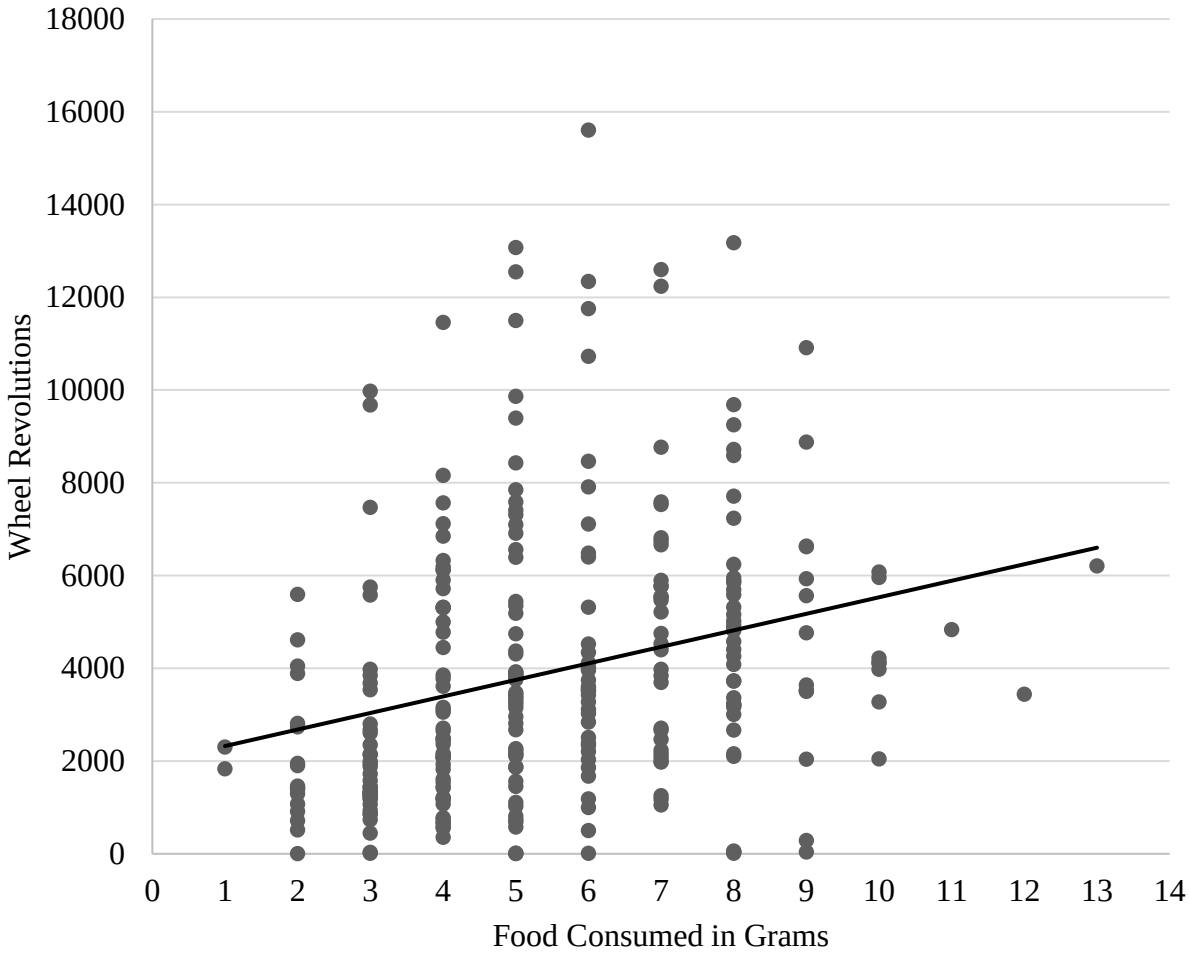


Figure 10 – Scatterplot for Daily Wheel Running per Food Consumed – This figure shows the amount of wheel running per gram of food consumed for all subjects (N = 32). It also includes the significant regression line that predicted wheel running based on food consumption, $F(1, 292) = 23.02$, $p < 0.001$, $r^2 = 0.07$. Wheel running is equal to 1966.92 + 365.44 (food consumed). For every gram of food consumed, wheel running increased by 365.44 revolutions.

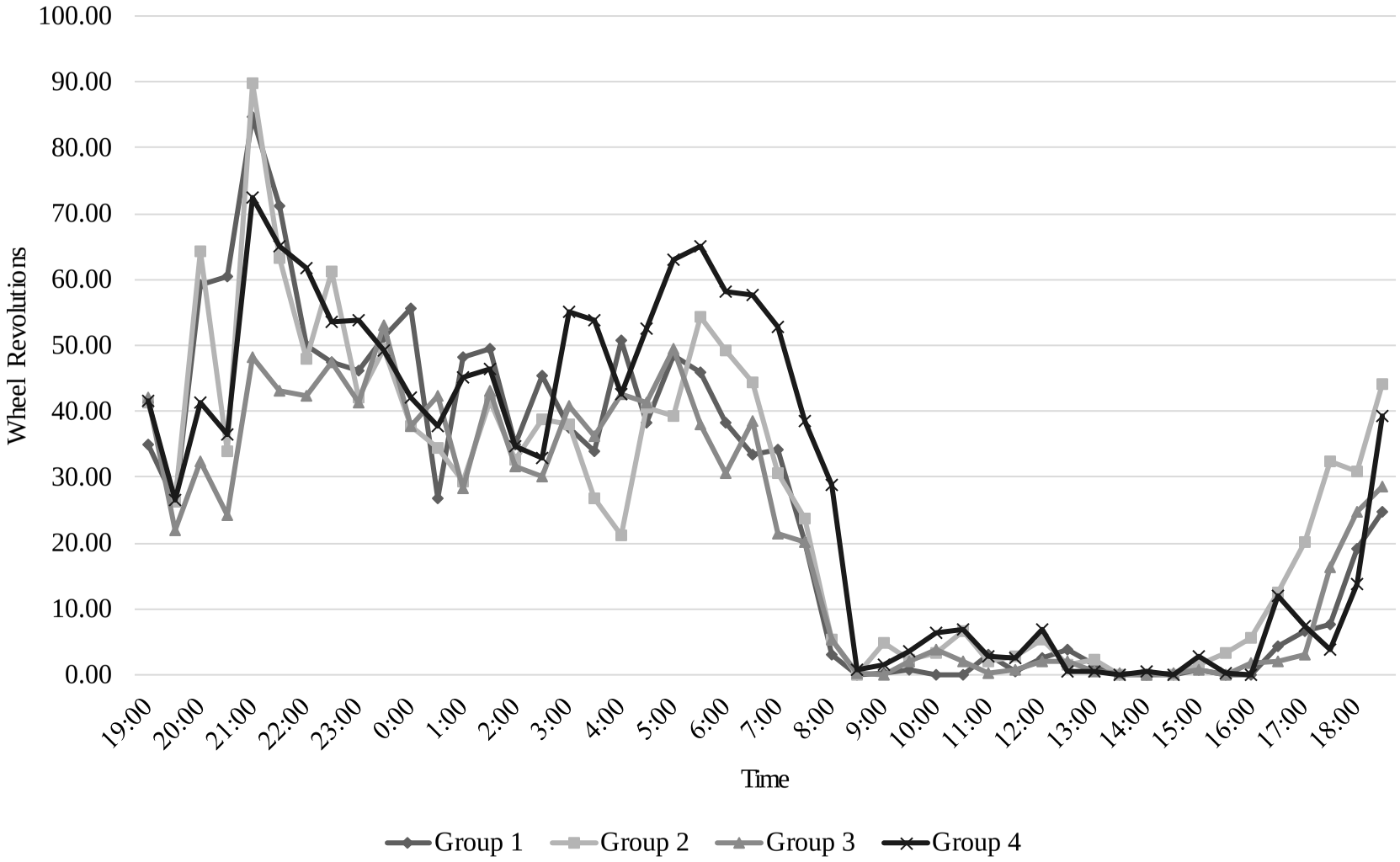


Figure 11 – Group Baseline Running by Half Hour – This figure shows the average wheel rotations for each group during the B days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

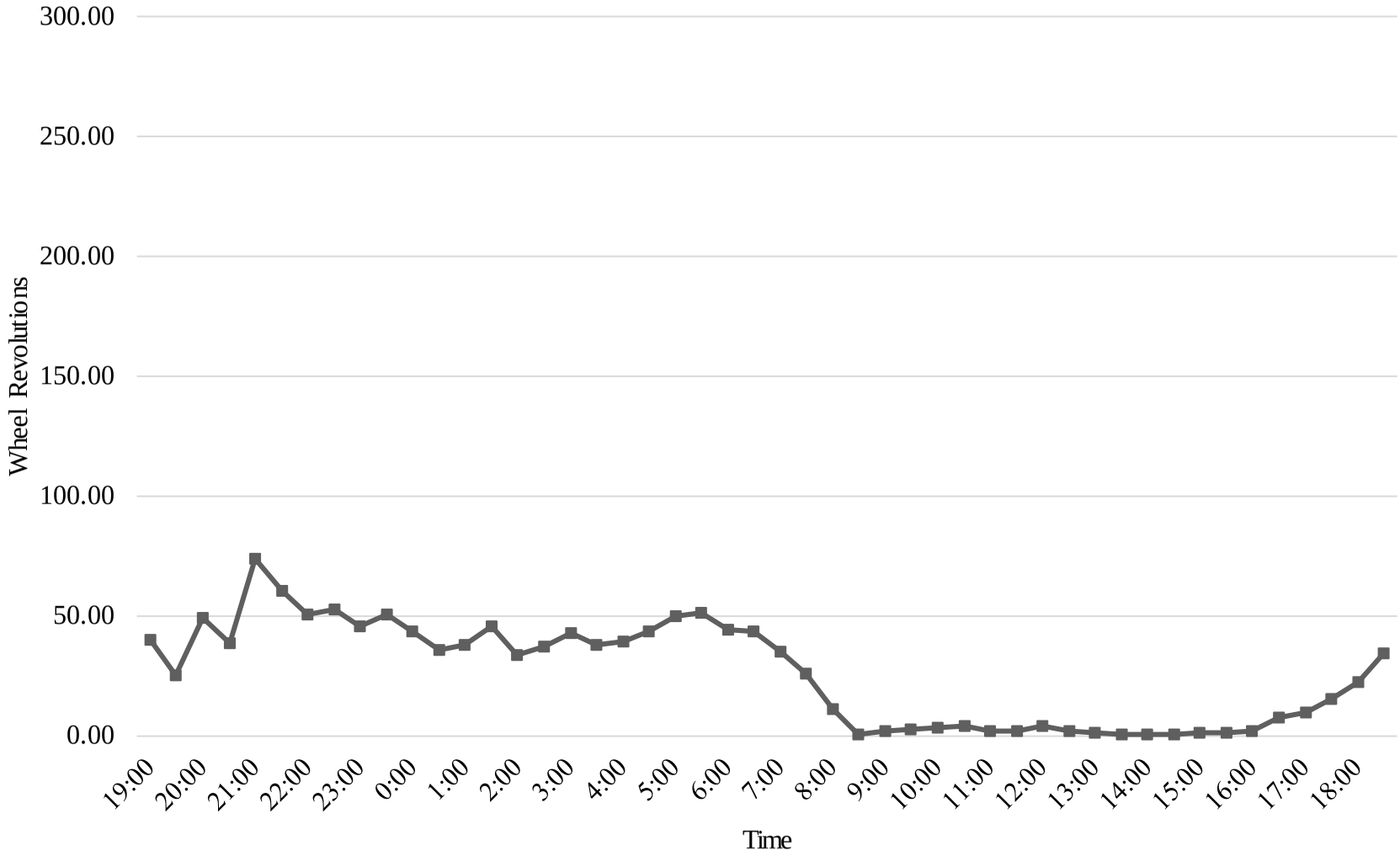


Figure 11 – Average Baseline Running by Half Hour – This figure shows the average wheel rotations for all the groups during B days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

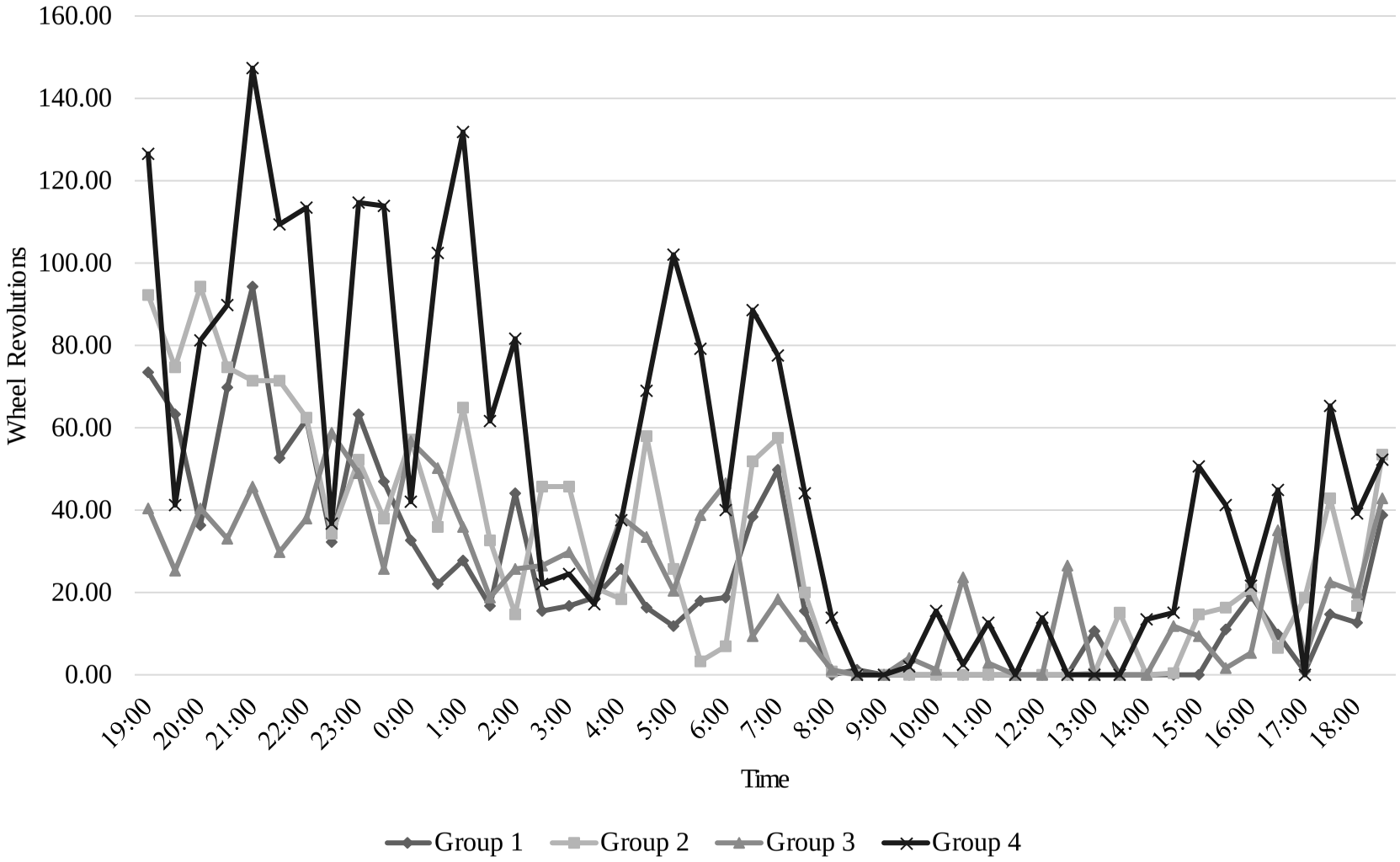


Figure 13 – Average Group T1 Running by Half Hour – This figure shows the average wheel rotations for each group during T1 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

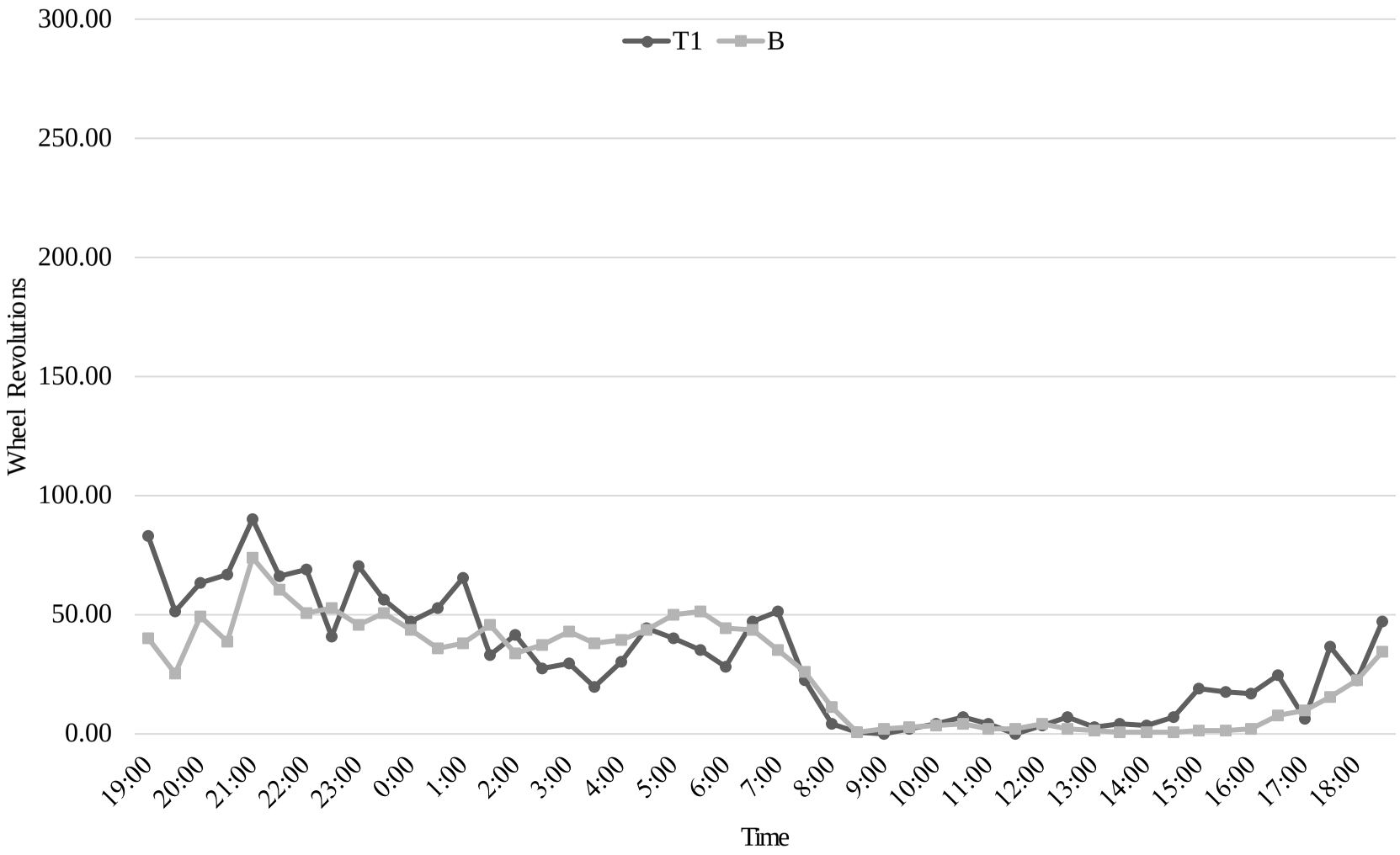


Figure 14 – Average T1 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T1 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

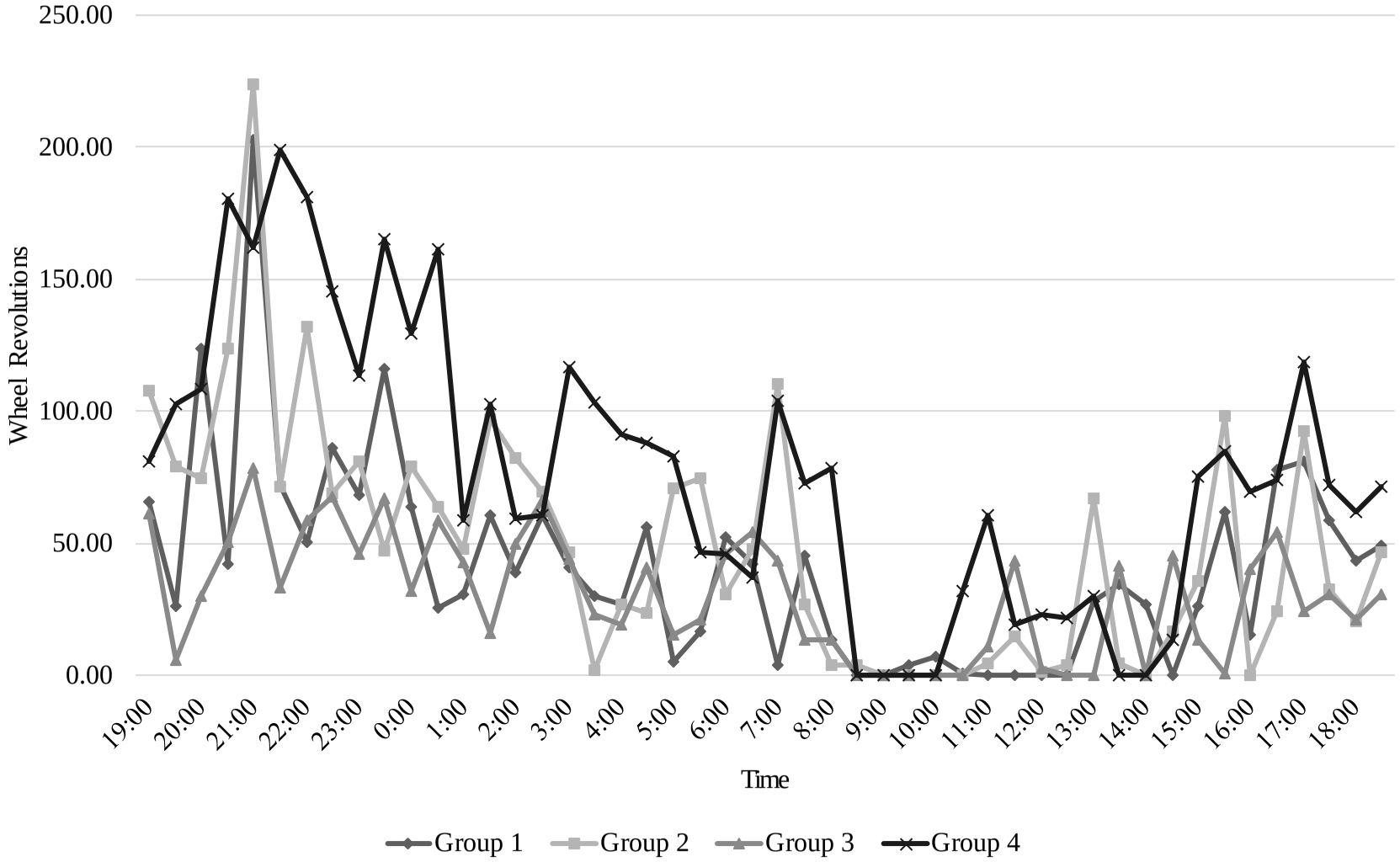


Figure 15 – Average Group T2 Running by Half Hour – This figure shows the average wheel rotations for each group during T2 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

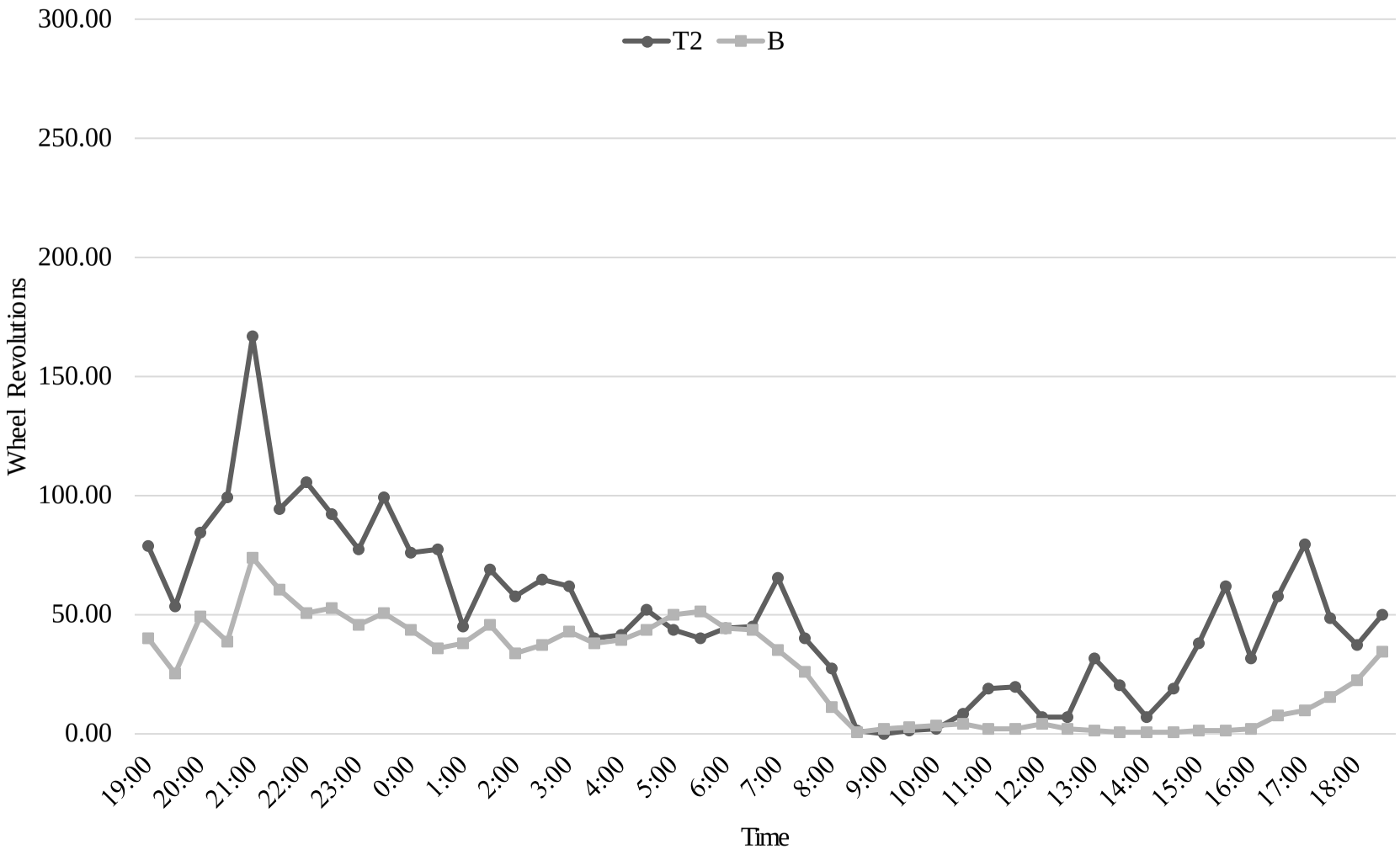


Figure 16 – Average T2 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T2 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

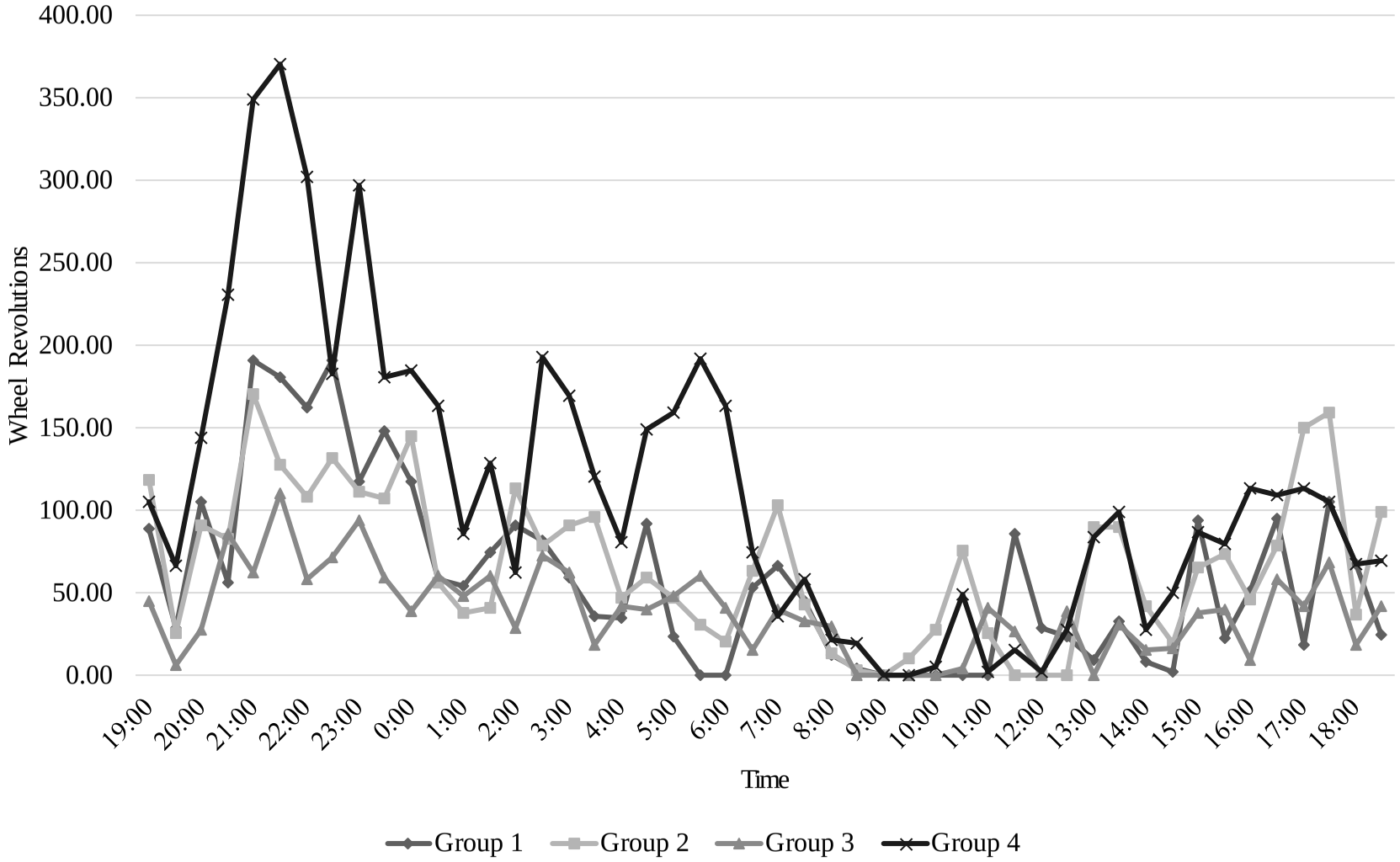


Figure 17 – Average Group T3 Running by Half Hour – This figure shows the average wheel rotations for each group during T3 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

Figure 18 – Average T3 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T3 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

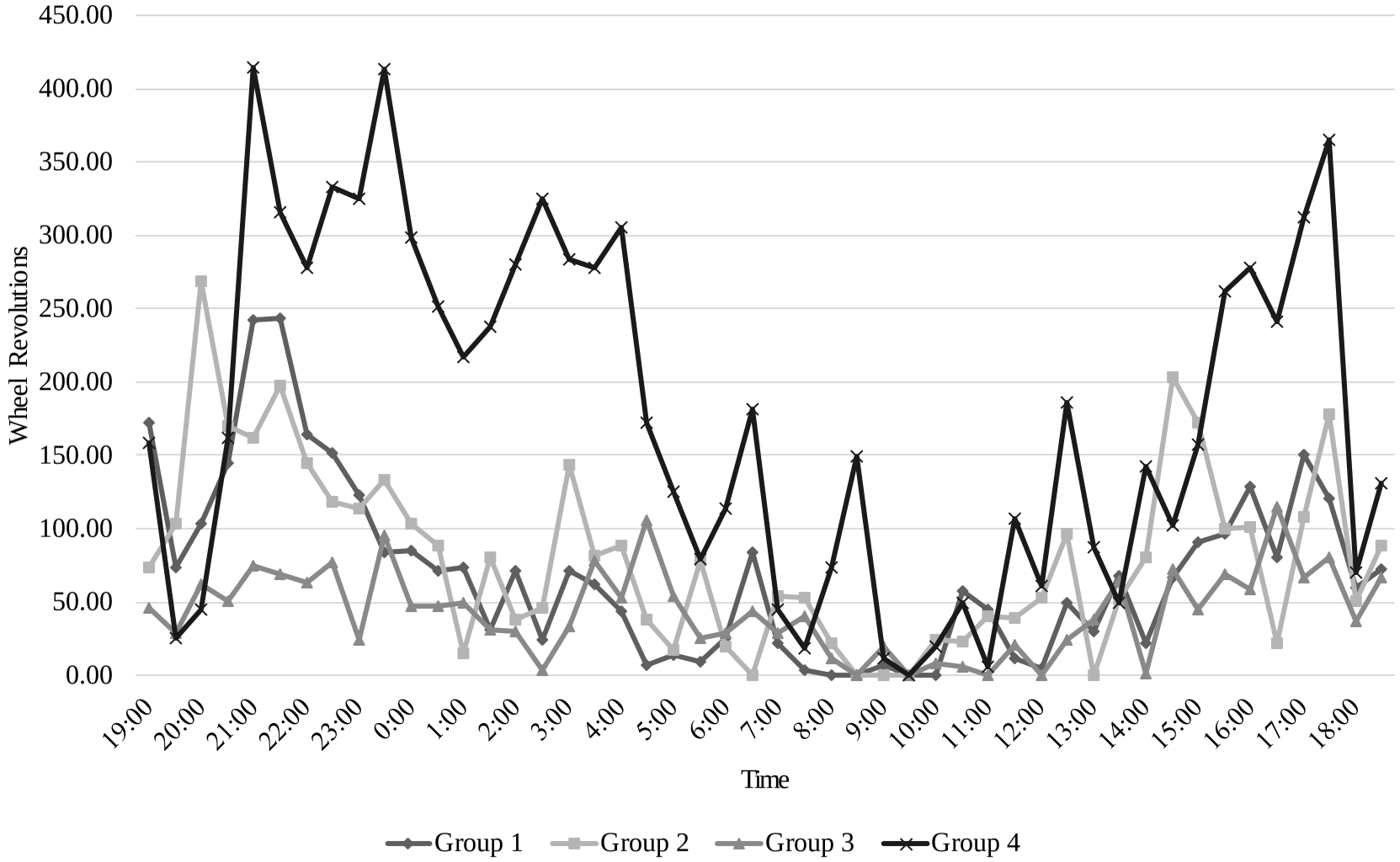


Figure 19 – Average Group T4 Running by Half Hour – This figure shows the average wheel rotations for each group during T4 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

Figure 20 – Average T4 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T4 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

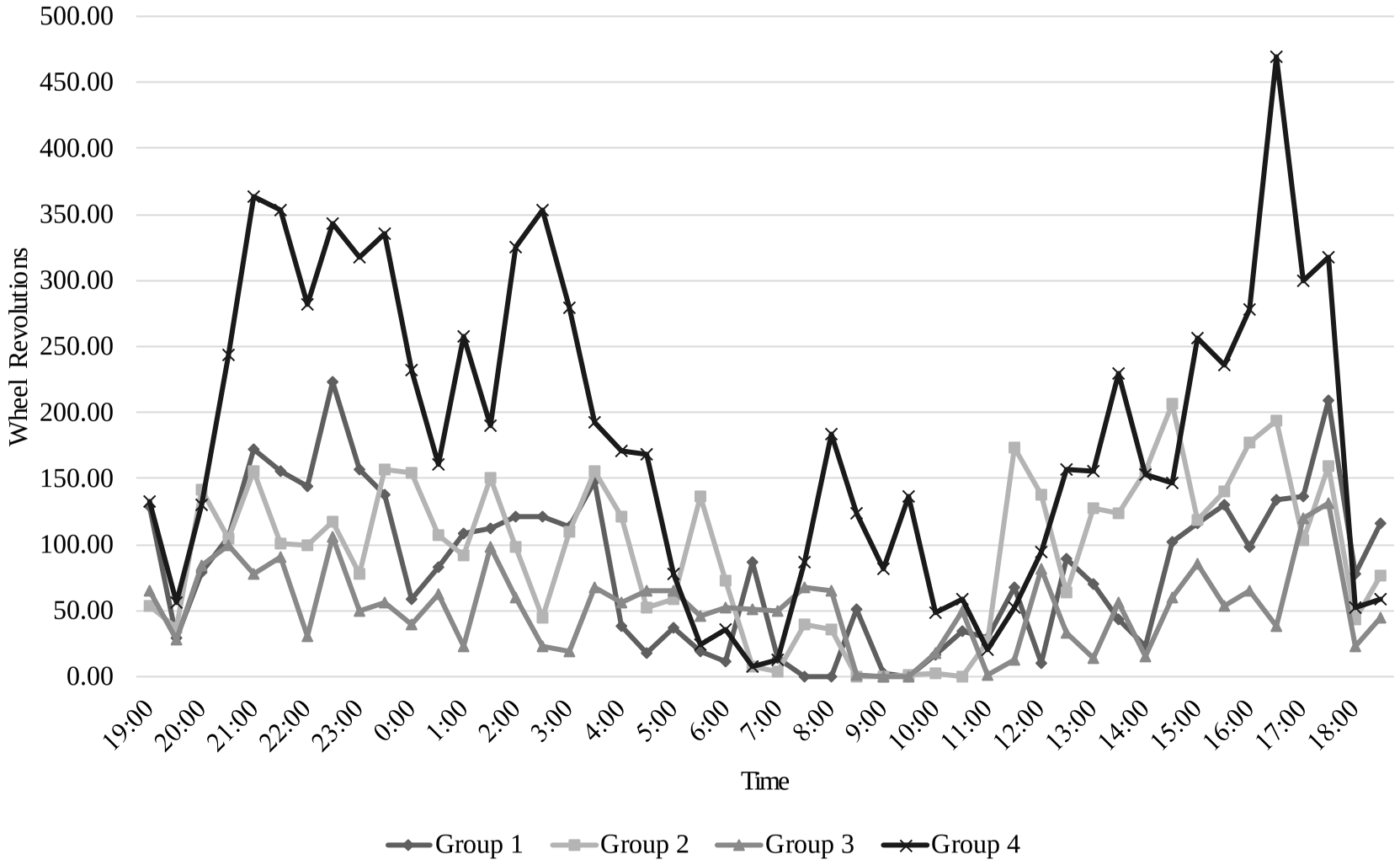


Figure 21 – Average Group T5 Running by Half Hour – This figure shows the average wheel rotations for each group during T5 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

Figure 22 – Average T5 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T5 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

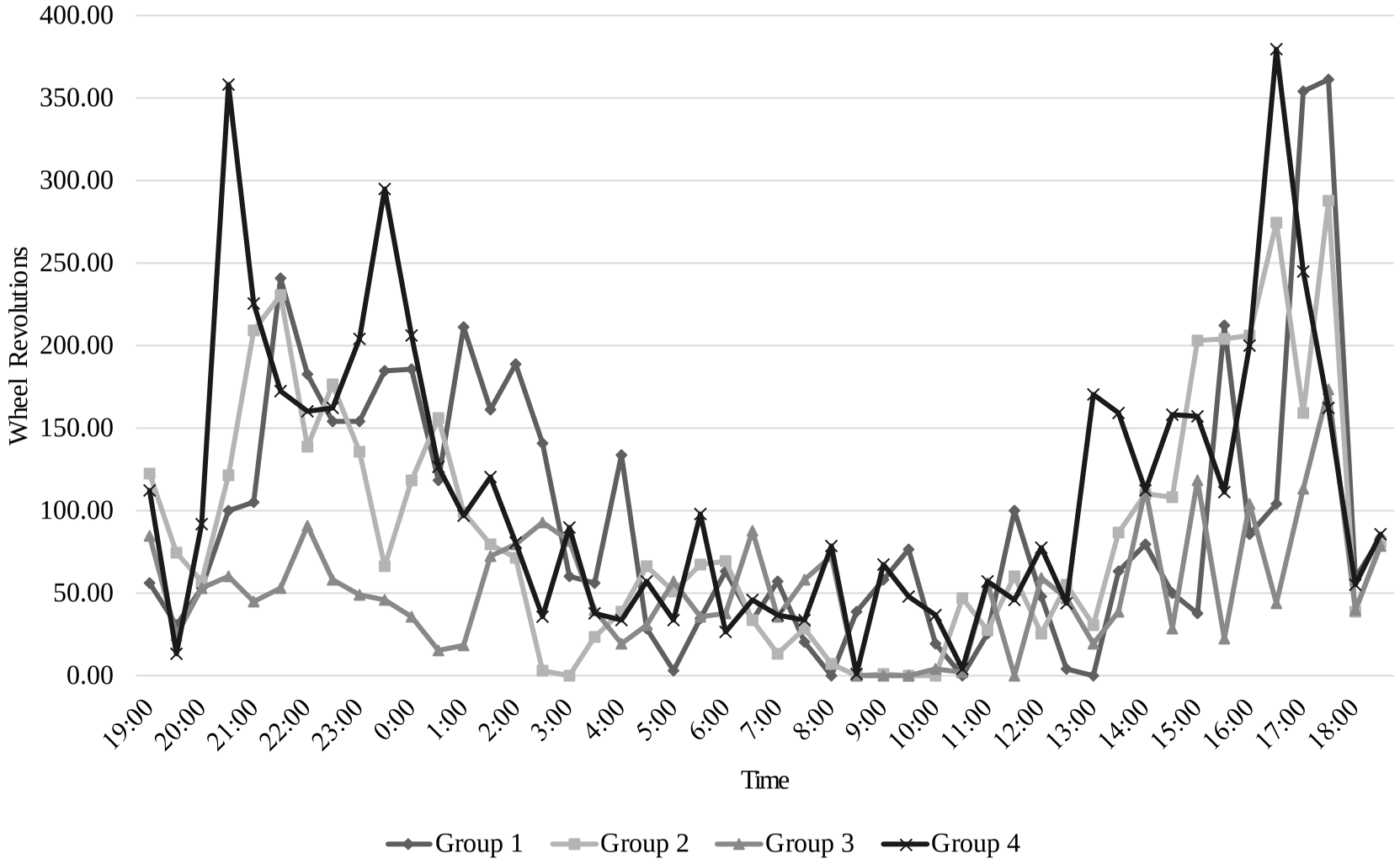


Figure 23 – Average Group T6 Running by Half Hour – This figure shows the average wheel rotations for each group during T6 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

Figure 24 – Average T6 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T6 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

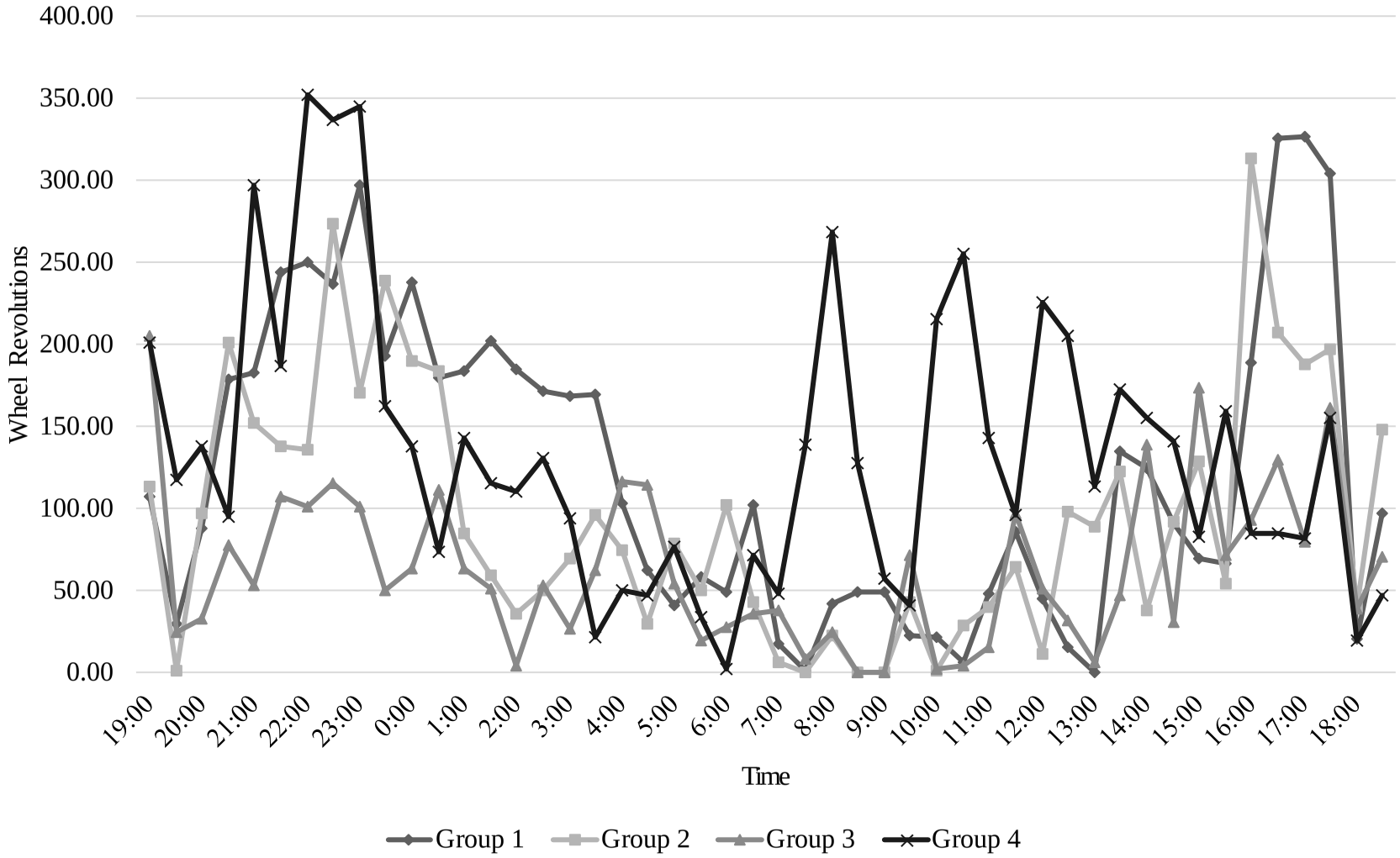


Figure 25 – Average Group T7 Running by Half Hour – This figure shows the average wheel rotations for each group during T7 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

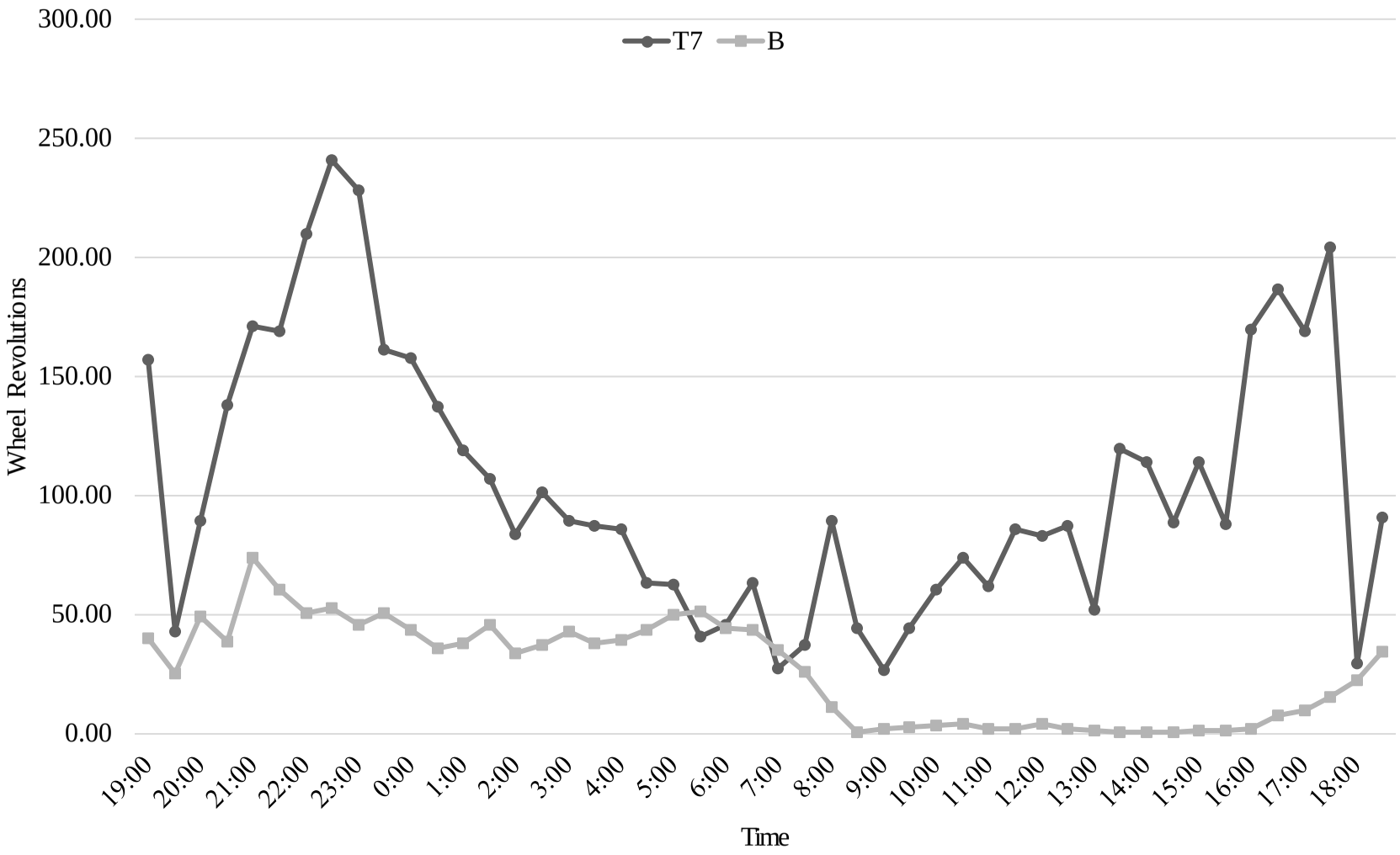


Figure 26 – Average T7 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T7 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

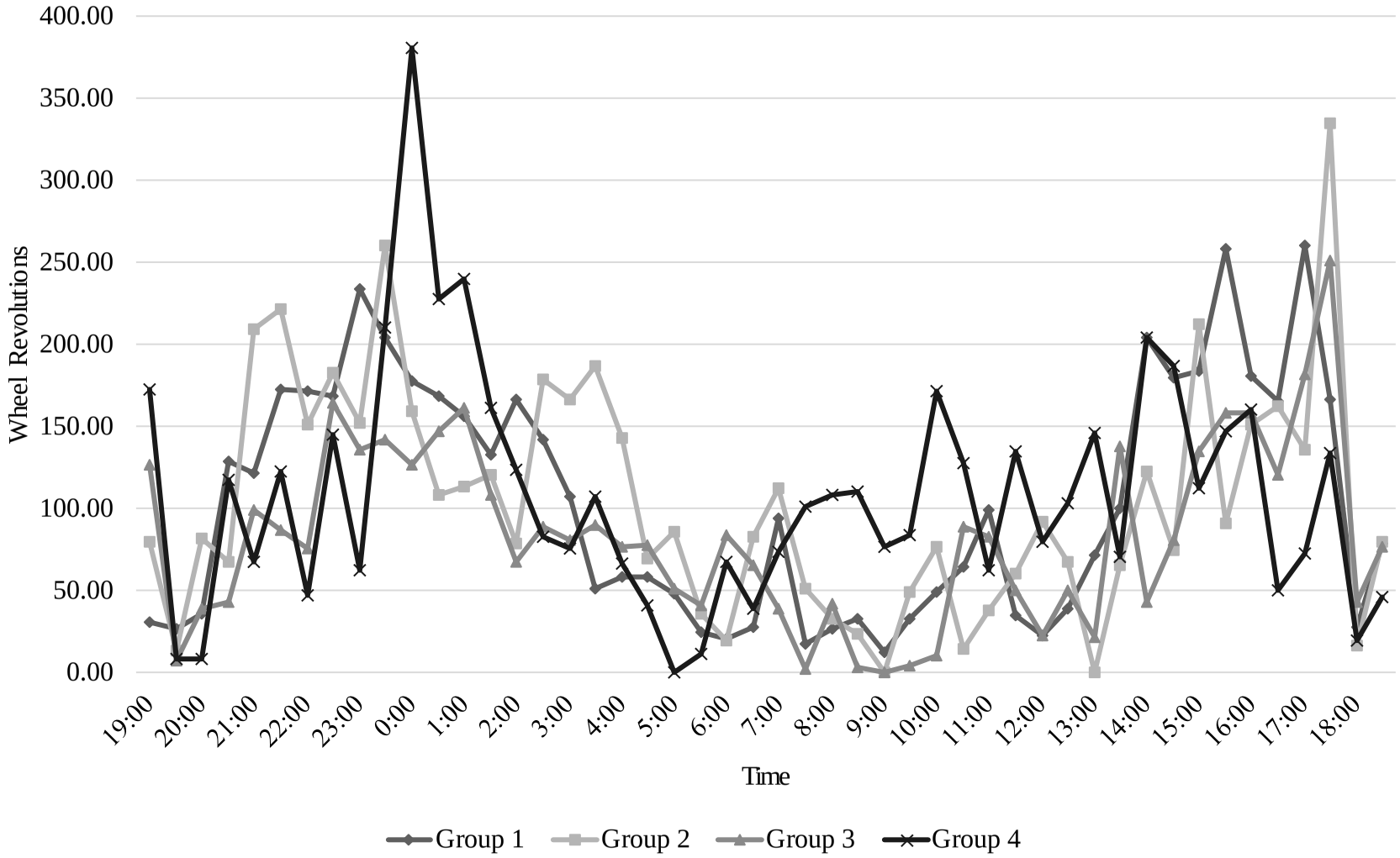
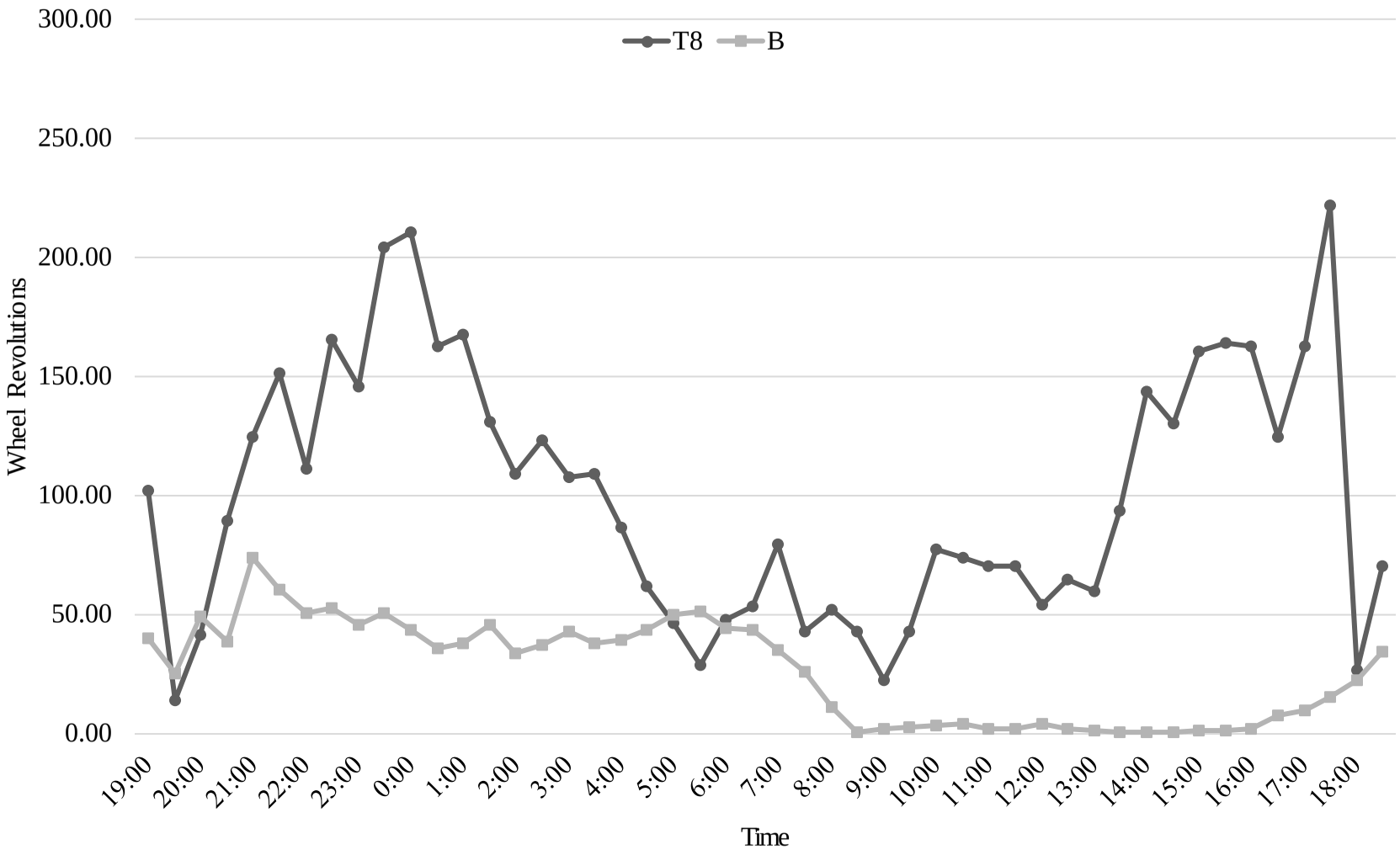


Figure 27 – Average Group T8 Running by Half Hour – This figure shows the average wheel rotations for each group during T8 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.



100

Figure 28 – Average T8 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T8 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

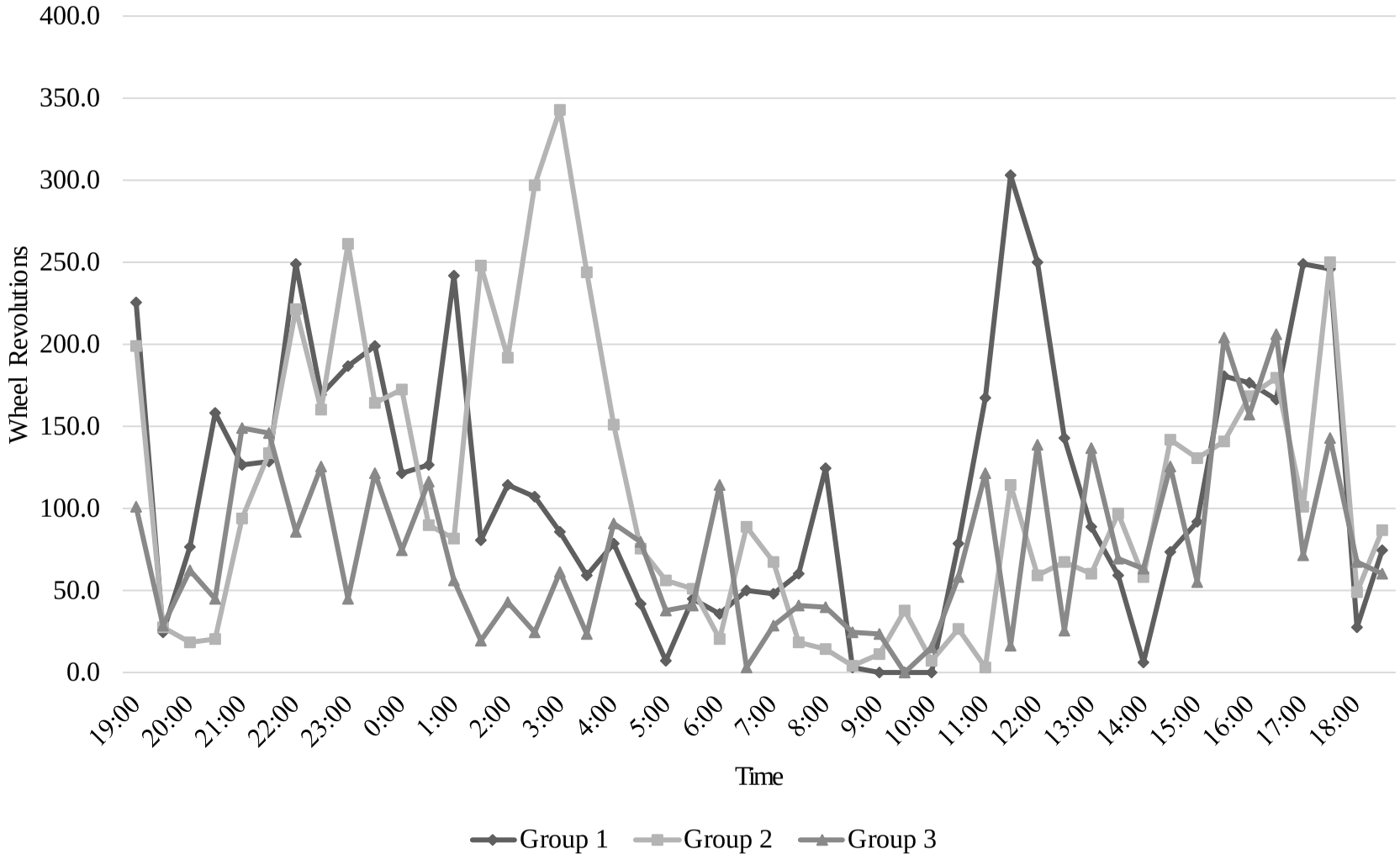


Figure 29 – Average Group T9 Running by Half Hour – This figure shows the average wheel rotations for each group during T9 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

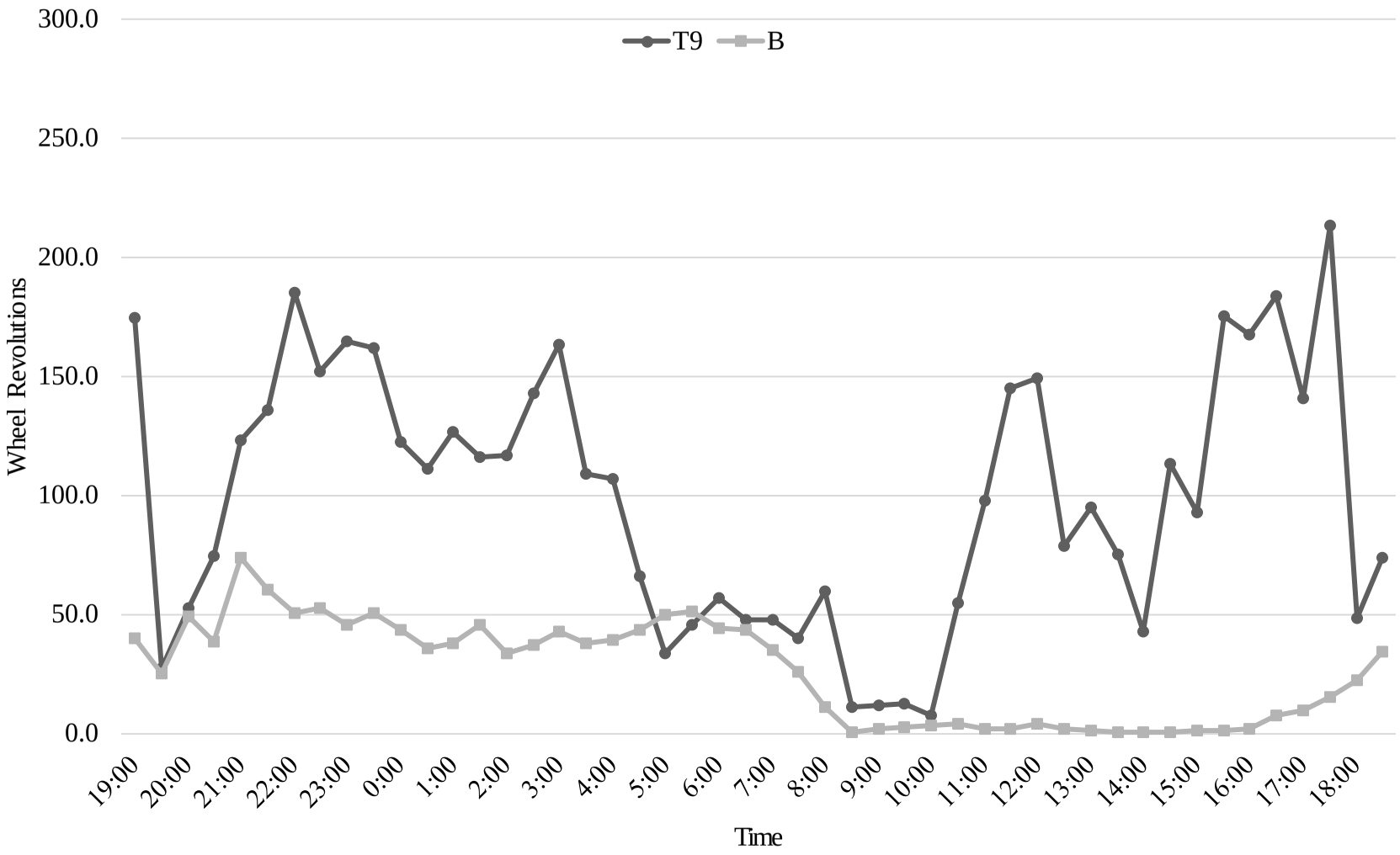


Figure 30 – Average T9 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T9 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

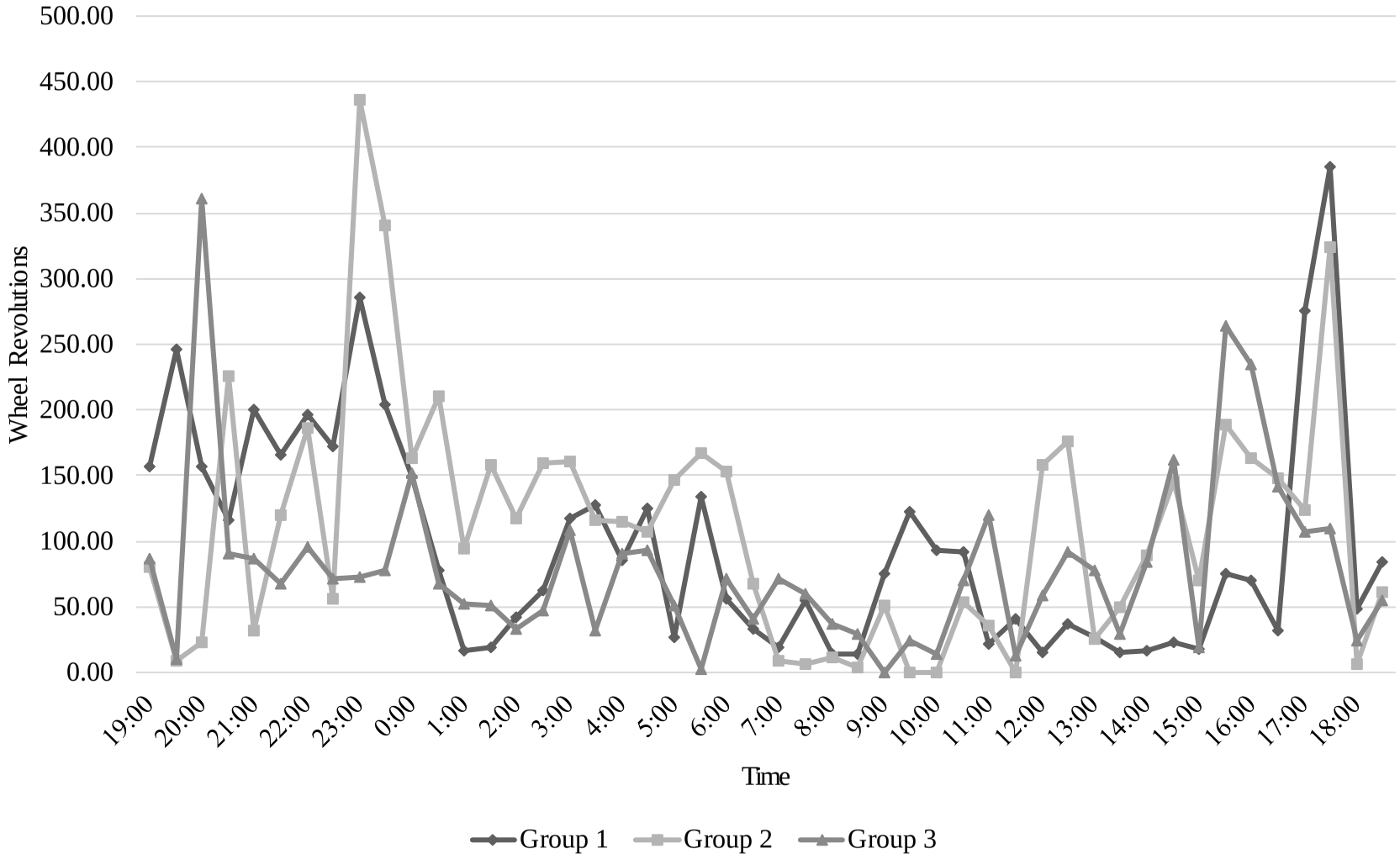


Figure 31 – Average Group T10 Running by Half Hour – This figure shows the average wheel rotations for each group during T10 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

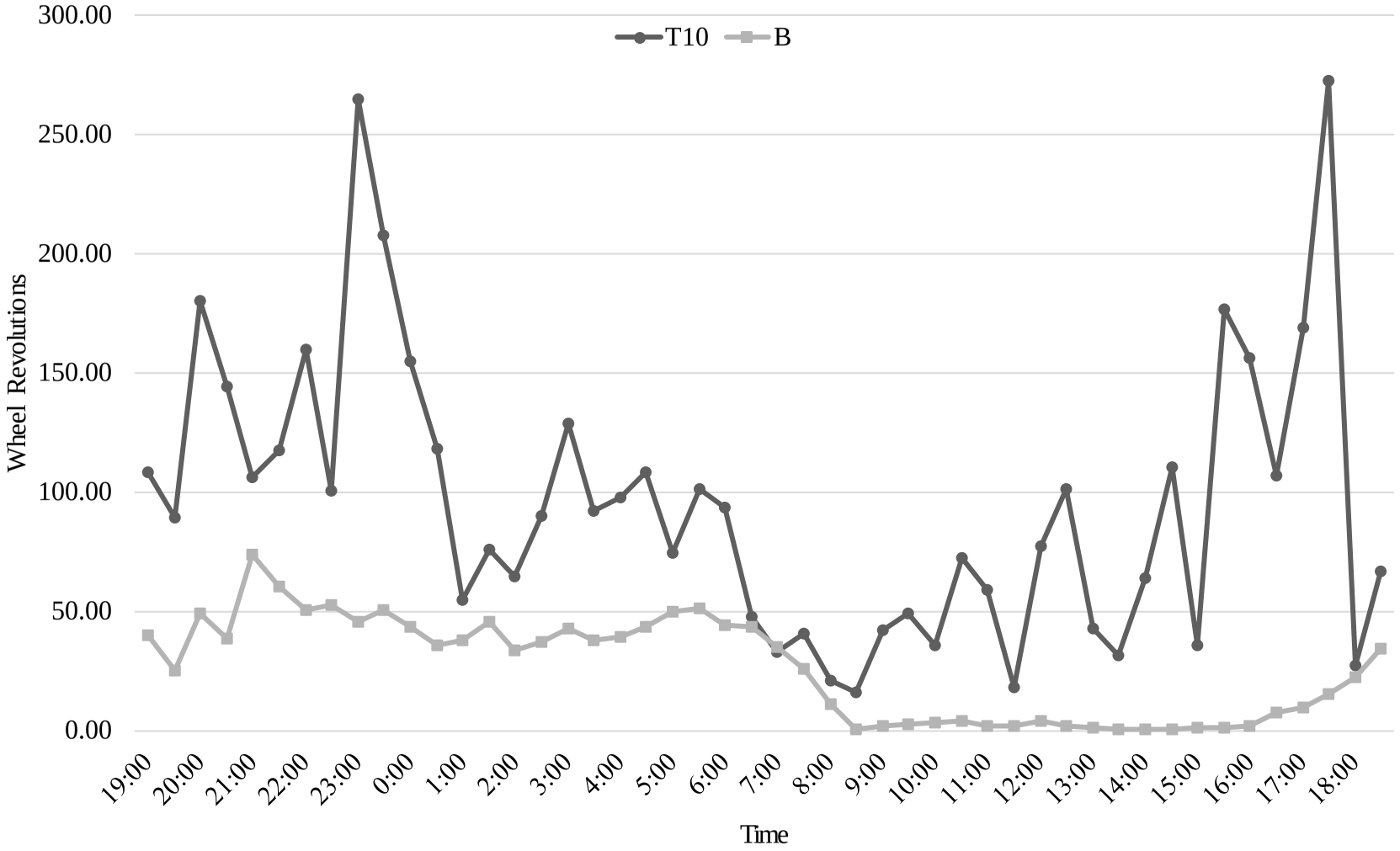


Figure 32 – Average T10 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T10 day. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours. The Baseline trend is also displayed for comparison.

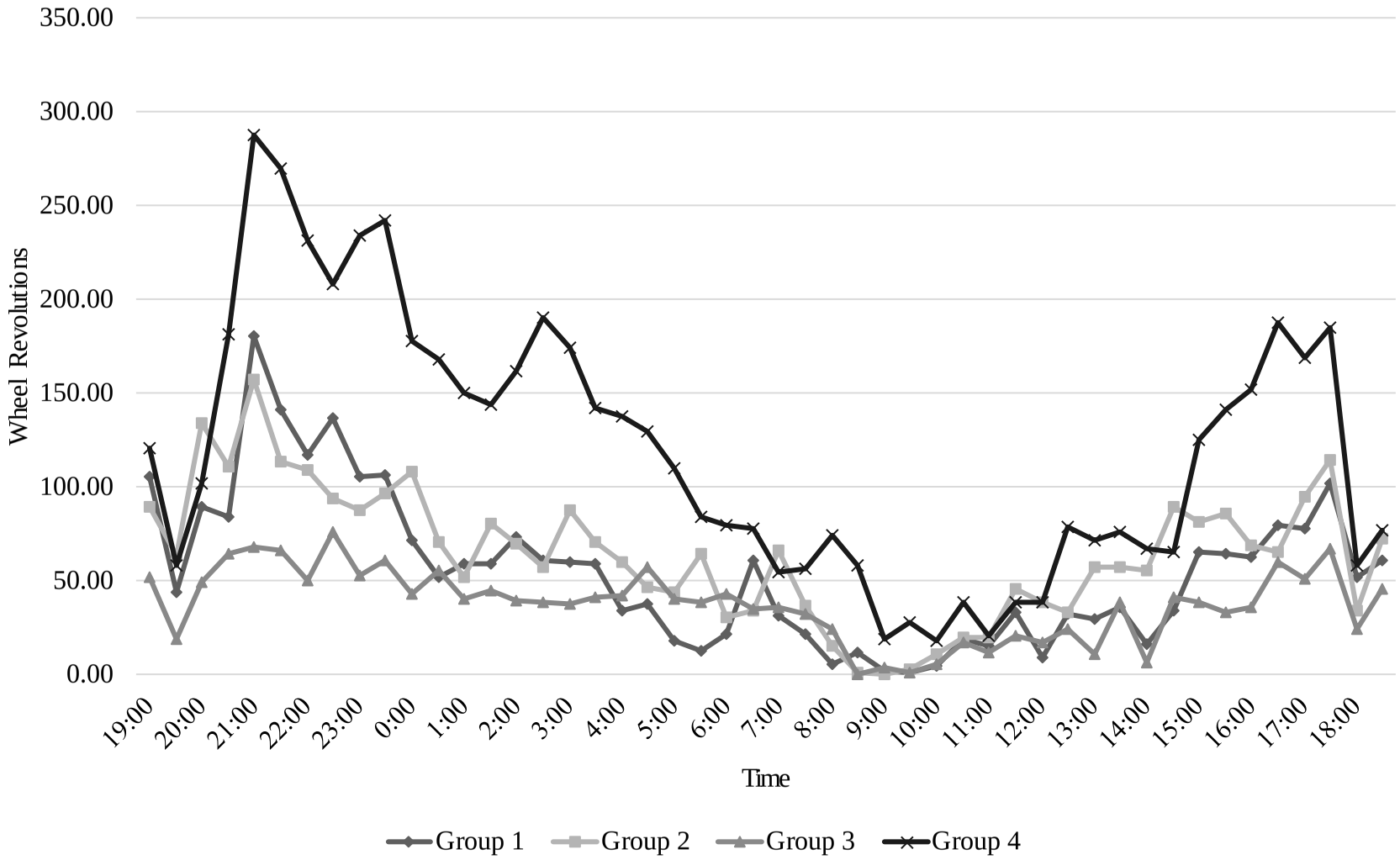


Figure 33 – Average Group T1 to T5 Running by Half Hour – This figure shows the average wheel rotations for each group during T1 through T5 days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

Figure 34 – Average T1 to T5 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T1 through T5 days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

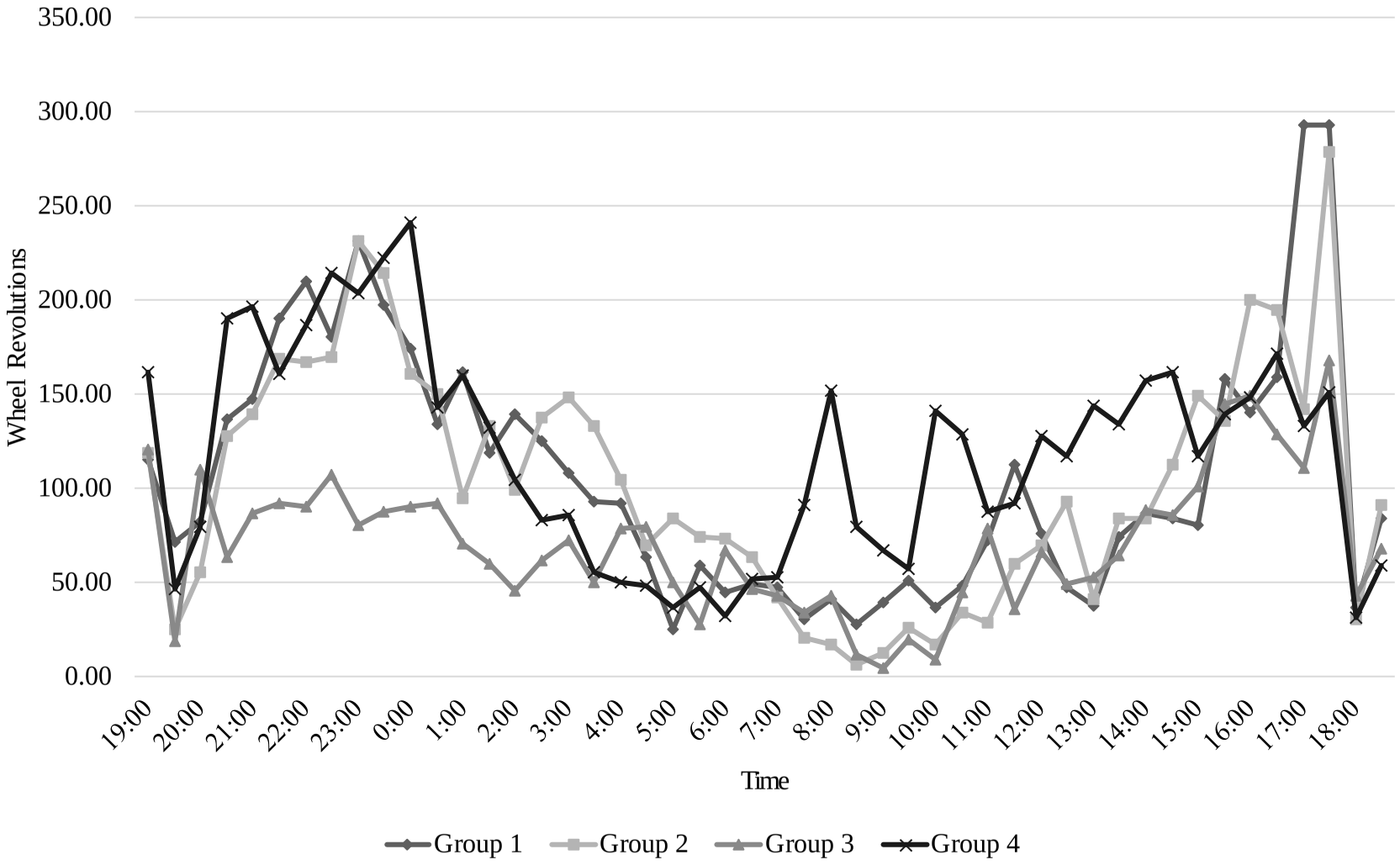


Figure 35 – Average Group T6 to T10 Running by Half Hour – This figure shows the average wheel rotations for each group during T6 through T10 days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

Figure 36 – Average T5 to T10 Running by Half Hour – This figure shows the average wheel rotations for all the groups during T5 through T10 days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

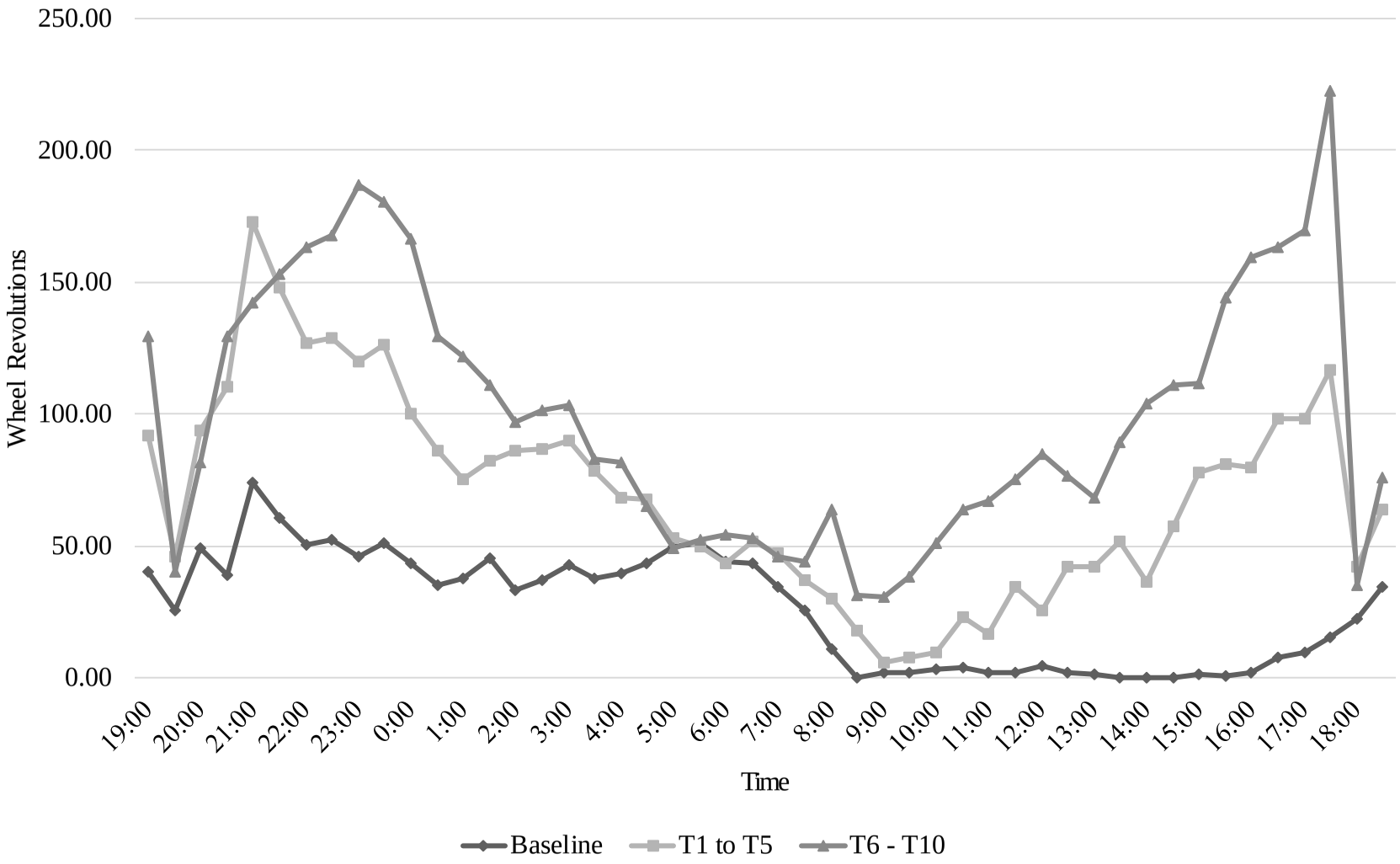


Figure 37 – Average Baseline, T1 to T5, and T6 to T10 Running by Half Hour – This figure shows the average wheel rotations for all the groups during B, T1 through T5, and T6 through T10 days. Average wheel revolutions are displayed by every 30 minutes starting at 1900 hours and ending at 1830 hours.

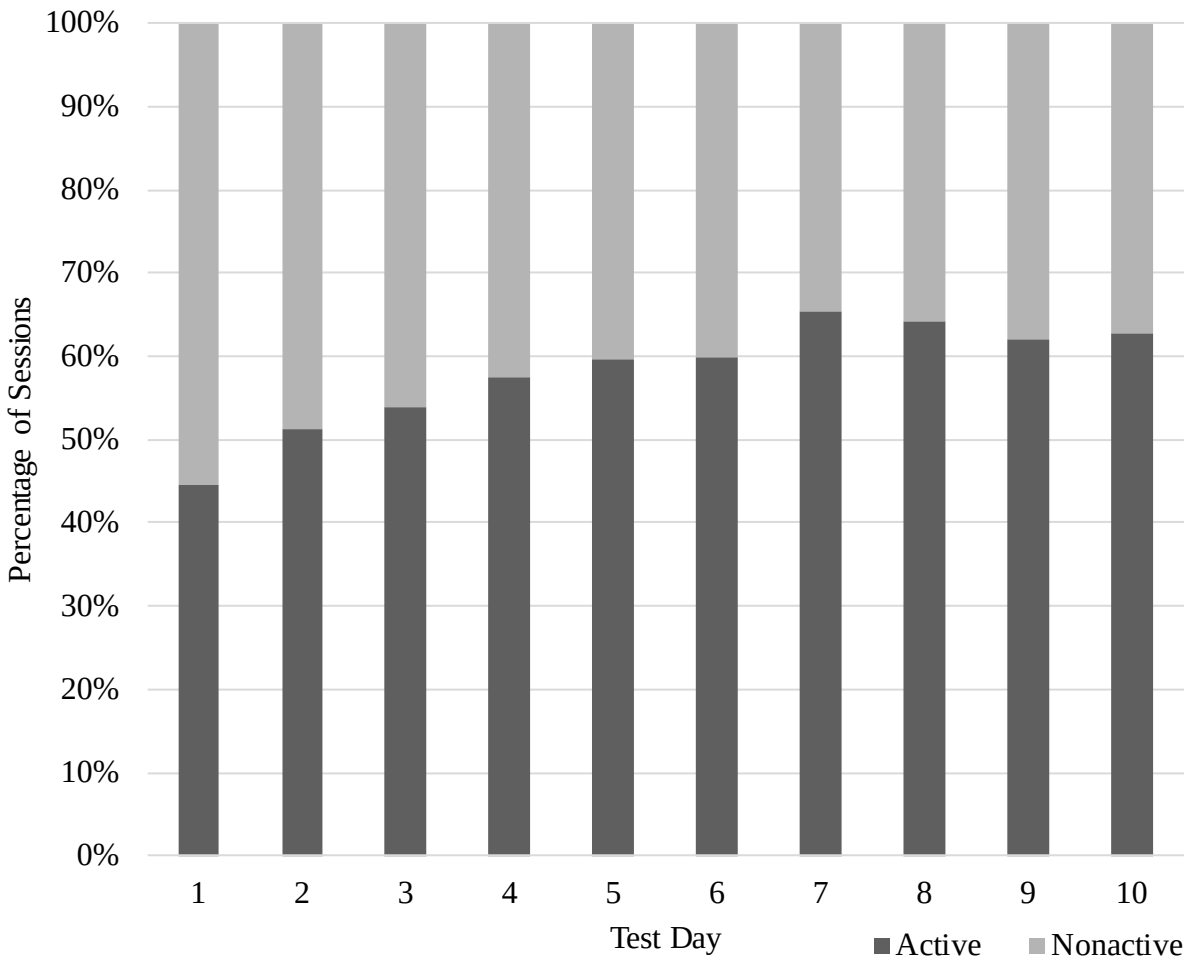


Figure 38 – Percentage of Activity by Test Day – This figure shows the percentage of activity and inactivity per T day. Baseline rates indicate an average of 45% activity.

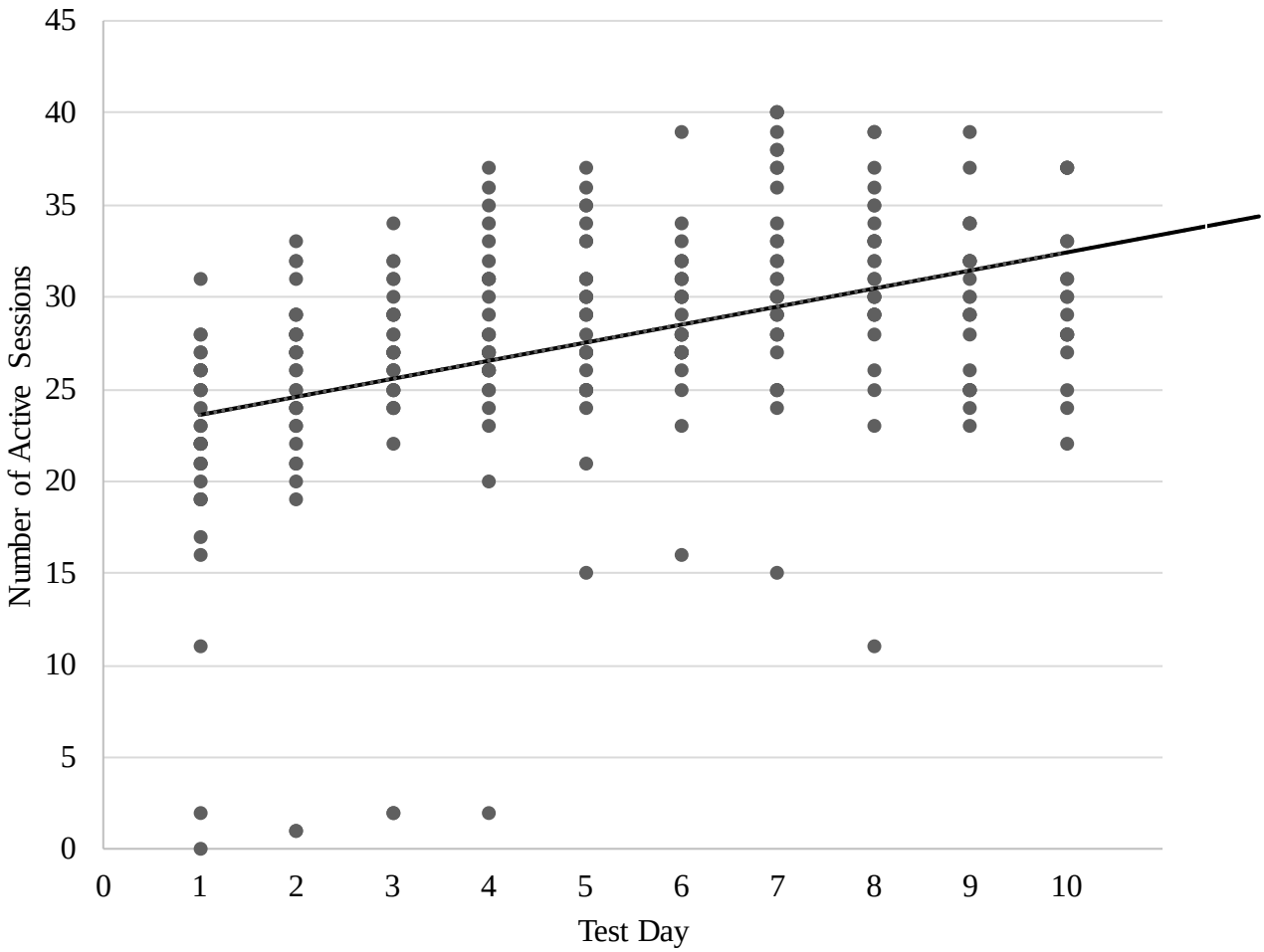


Figure 39 – Scatterplot of Activity Sessions by Test Day – This figure shows the scatterplot of activity sessions for each T day for all subjects (N = 32). It also includes the significant regression line that predicted activity based on T day, $F(1, 252) = 63.62$, $p < 0.001$, $r^2 = 0.18$. Activity sessions is equal to 22.67 + 0.98 (T Day). For every T Day, Activity sessions increased by 0.98 sessions every T day.

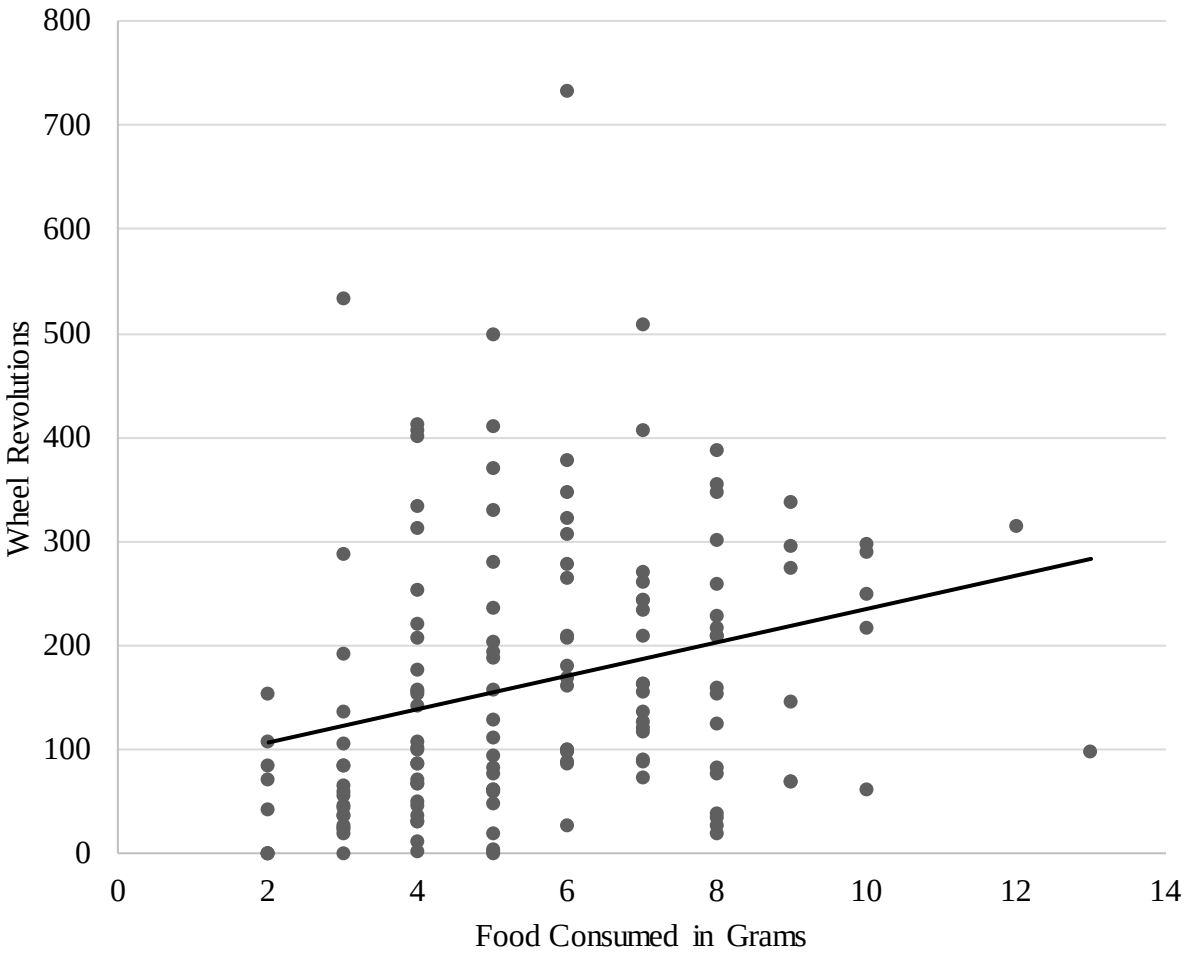


Figure 40 – Scatterplot for Wheel Running During Feeding Hour per Gram of Food Consumed – This figure shows the amount of wheel running per gram of food consumed during the one hour feeding period for the animals in the Unlocked condition (N =16). It also includes the significant regression line that predicted wheel running based on food consumption, $F(1, 143) = 11.44$, $p = 0.001$, $r^2 = 0.07$. Wheel running during the feeding hour is equal to 74.95 + 16.09 (food consumed). For every gram of food consumed, wheel running increased by 16.09 revolutions.

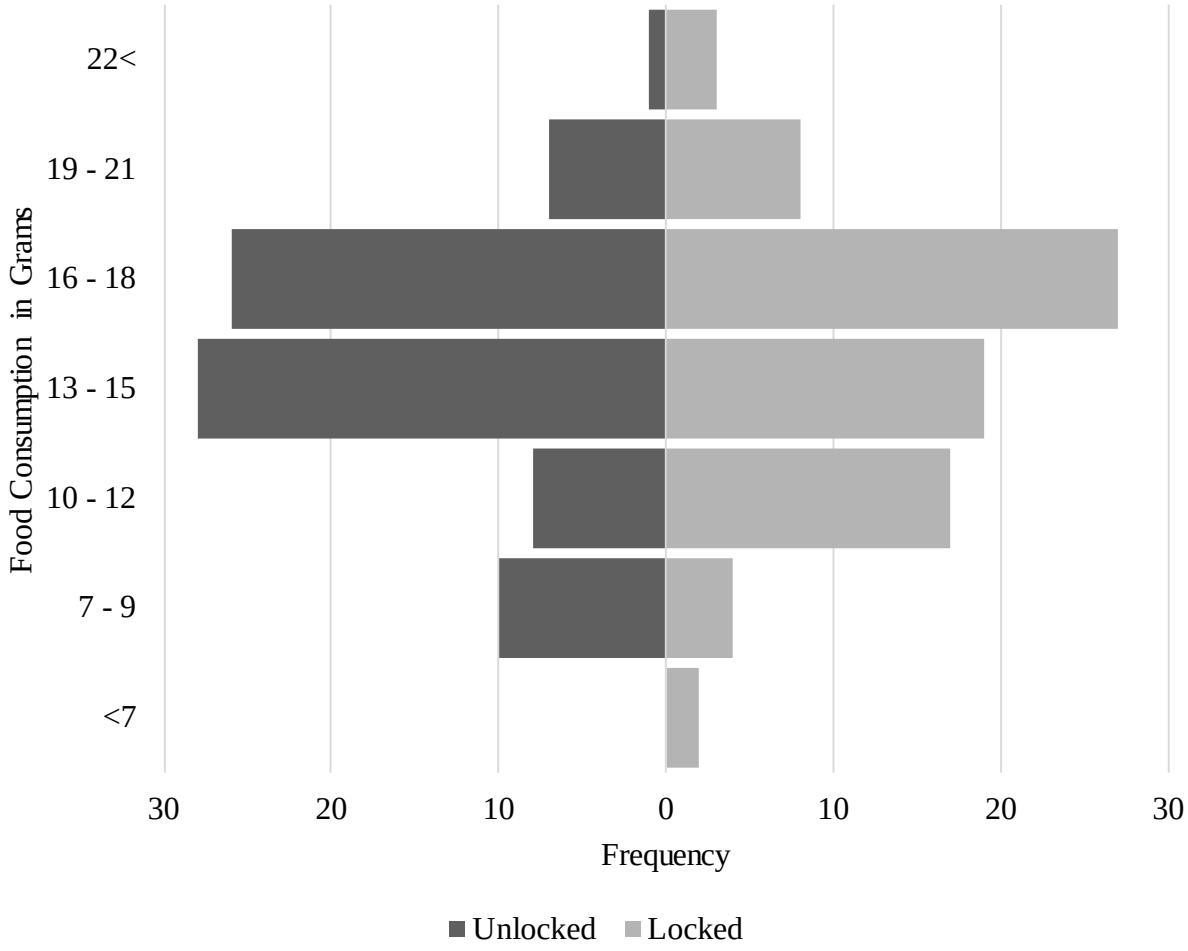


Figure 41 – Comparative Histogram of Baseline Food Consumption for Locked and Unlocked Groups – This figure shows the histogram of food consumption in grams during baseline. These groups did not differ from each other, $t(158) = 0.46$, $p = 0.65$.

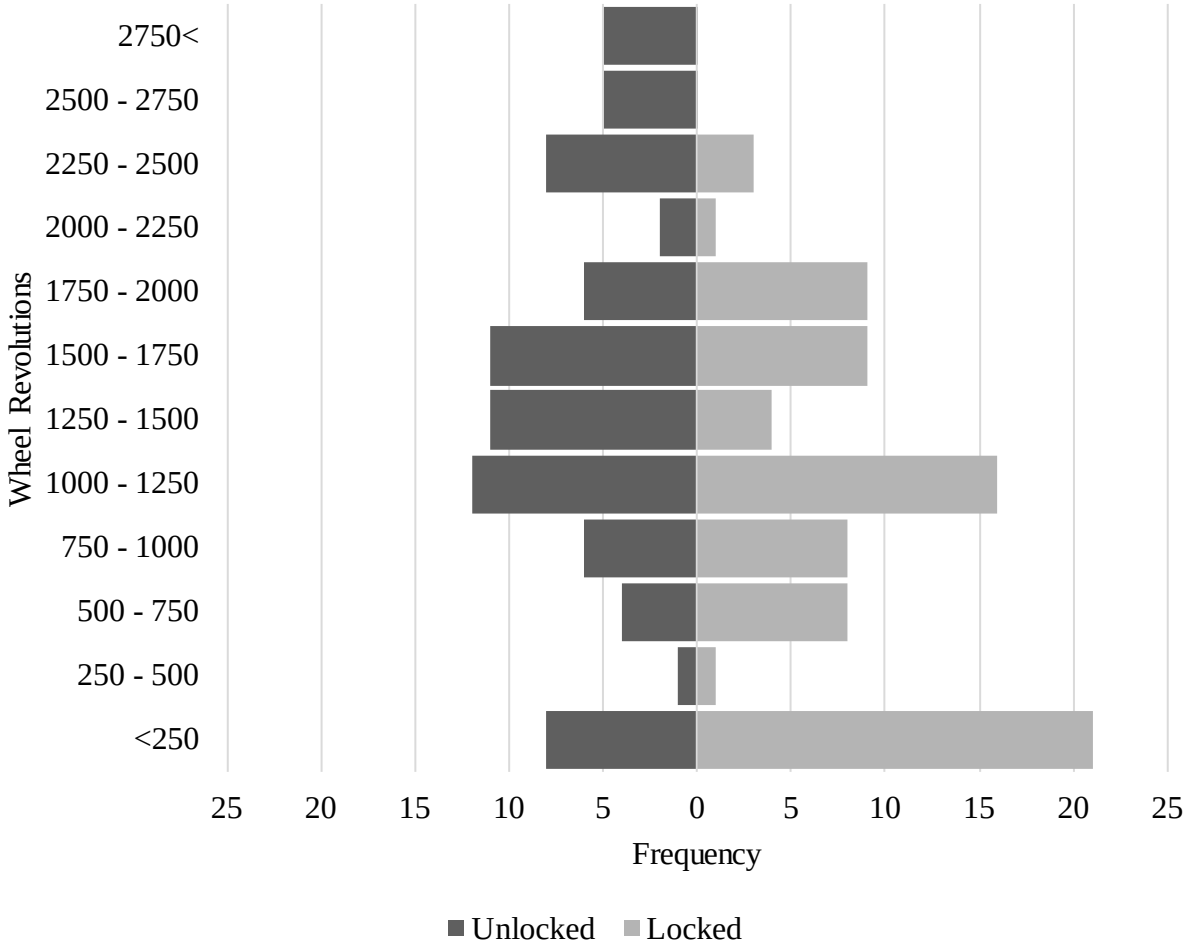


Figure 42 – Comparative Histogram of Baseline Wheel Running for Locked and Unlocked Groups - This figure shows the histogram of wheel running in grams during baseline. These groups did significantly differ from each other, $t(157) = -4.39$, $p < 0.001$, *Cohen's d* = 0.70.

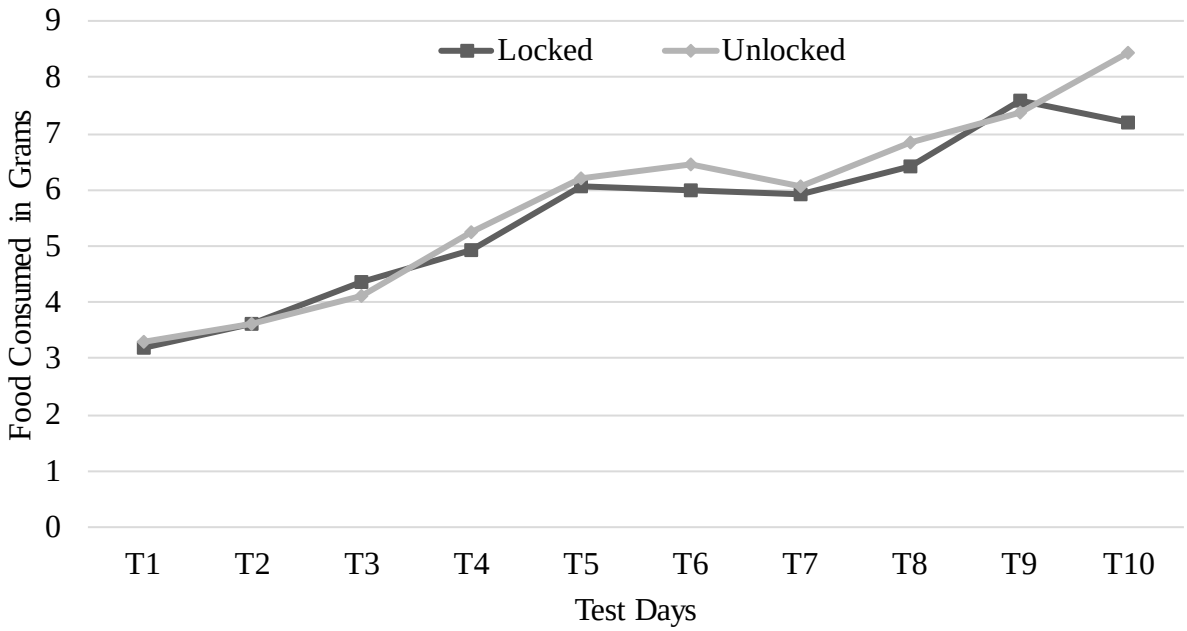


Figure 43 – Food Consumption by Test Day for Locked and Unlocked Groups – This figure shows the comparison of Locked and Unlocked groups on food consumed. No main effect of Lock ($F(1, 7) = 1.21$, $p = 0.31$) or the interaction of Lock X Days ($F(9, 63) = 0.58$, $p = 0.81$) was found. A main effect of Days ($F(9, 63) = 28.55$, $p < 0.001$, *partial* $\eta^2 = 0.15$) was found.

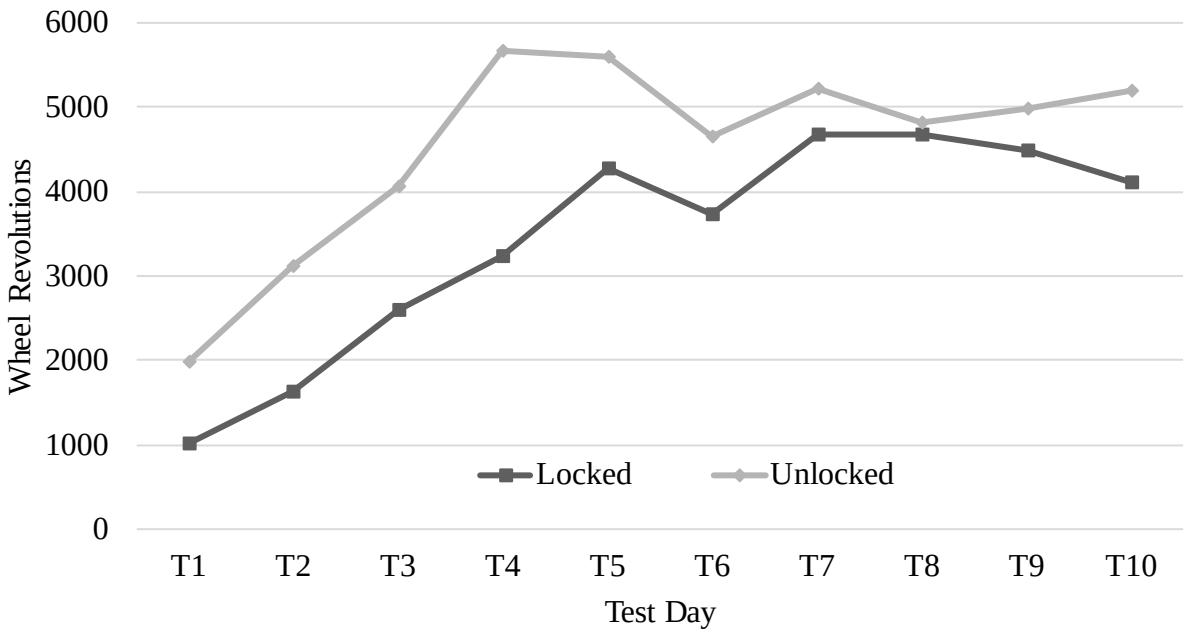


Figure 44 – Wheel Running by Test Day for Locked and Unlocked Groups – This figure shows the comparison of Locked and Unlocked groups on wheel running. No main effect of Lock ($F(1, 7) = 3.33$, $p = 0.11$) or the interaction of Lock X Days ($F(9, 63) = 0.92$, $p = 0.51$) was found. A main effect of Days ($F(9, 63) = 18.34$, $p < 0.001$, *partial* $\eta^2 = 0.72$) was found.

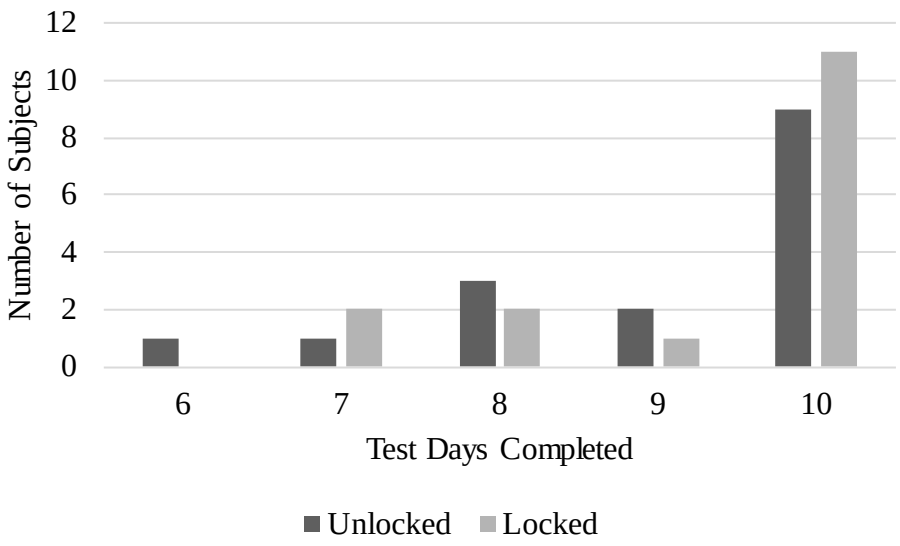


Figure 45 – Days Completed by Condition – This figure indicates the number of subjects that completed 6, 7, 8, 9, or 10 T days. No subjected completed less than 5 days, and the protocol ended on the 10th day.

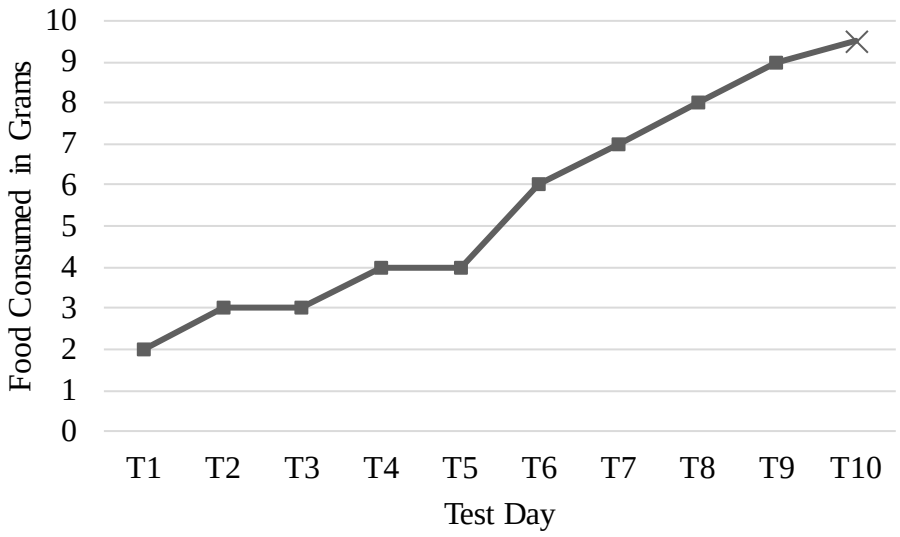


Figure 46 – Rat 5 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 5 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 5 for the regression line used to determine the missing data.

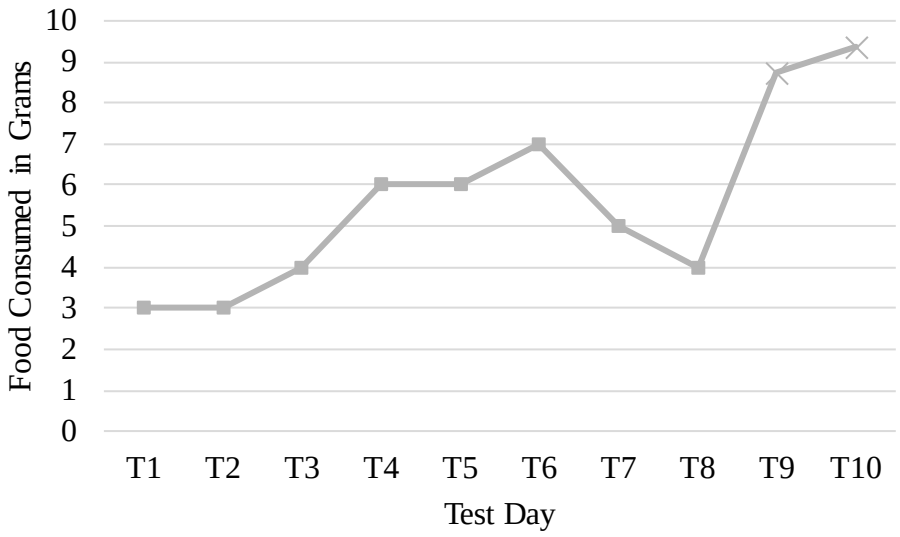


Figure 47 – Rat 7 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 7 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

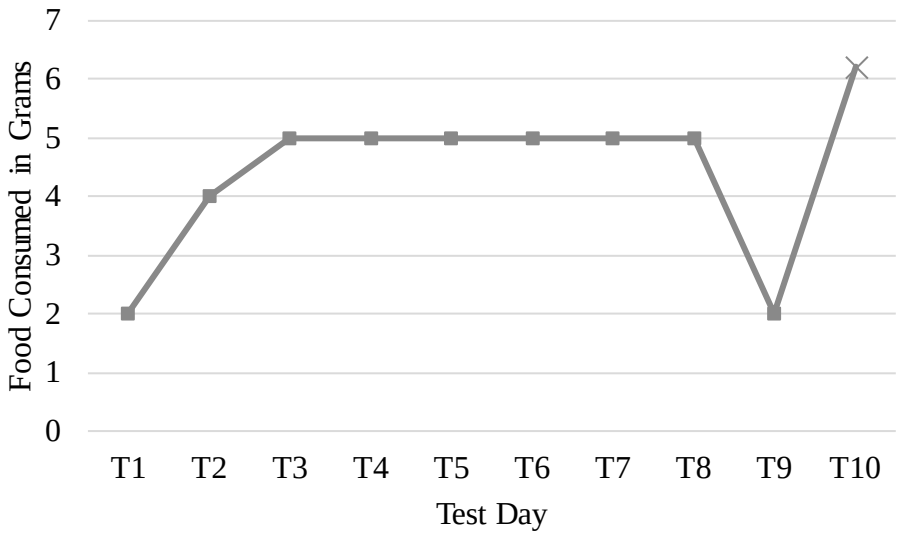


Figure 48 – Rat 13 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 13 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 5 for the regression line used to determine the missing data.

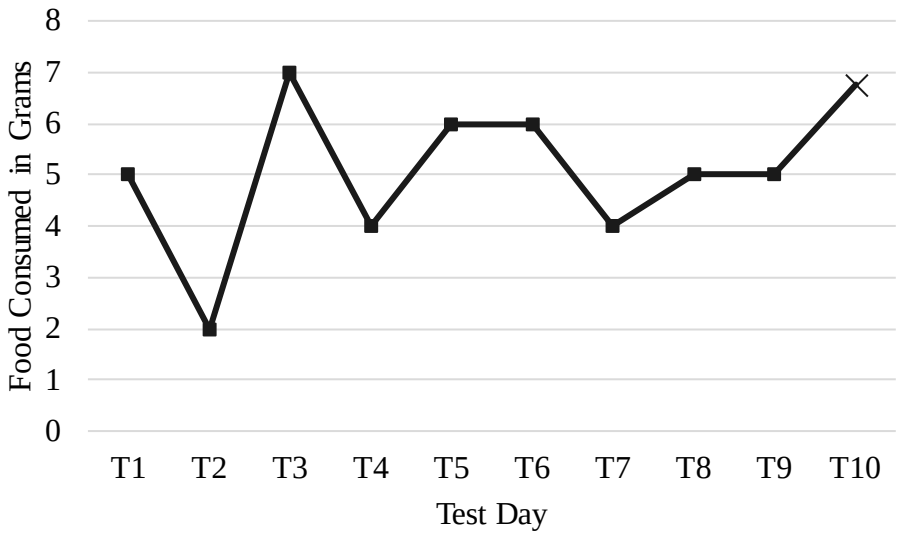


Figure 49 – Rat 17 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 17 with extrapolated data. The extrapolated points are indicated with an “X” on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

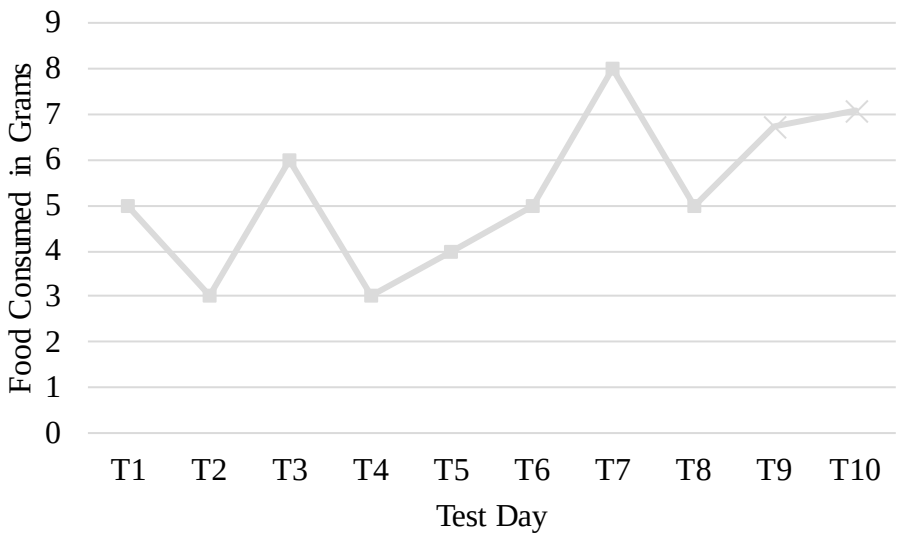


Figure 50 – Rat 25 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 25 with extrapolated data. The extrapolated points are indicated with an “X” on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

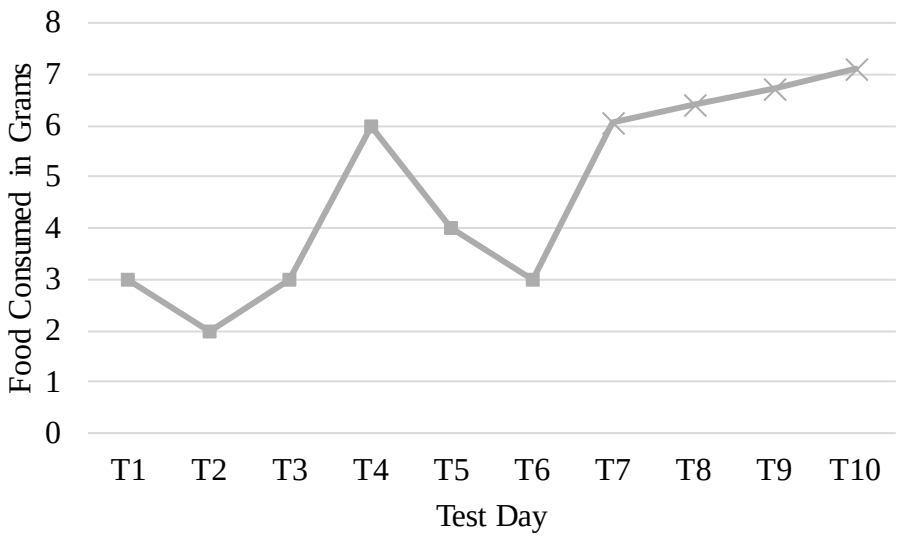


Figure 51 – Rat 26 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 26 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

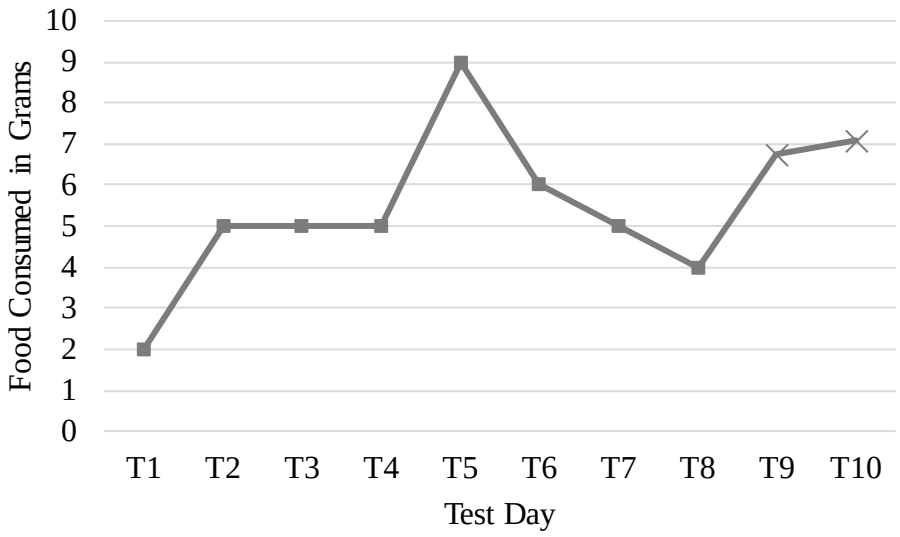


Figure 52 – Rat 27 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 27 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

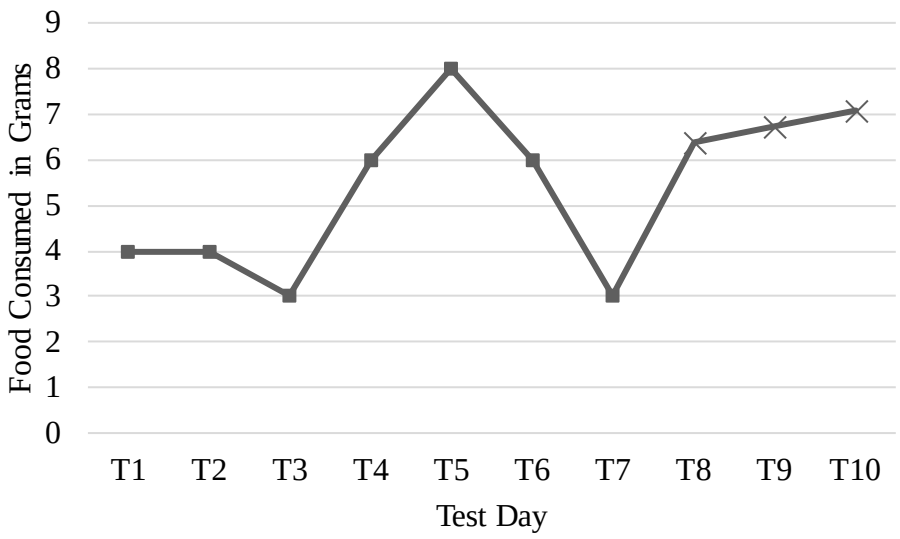


Figure 53 – Rat 28 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 28 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

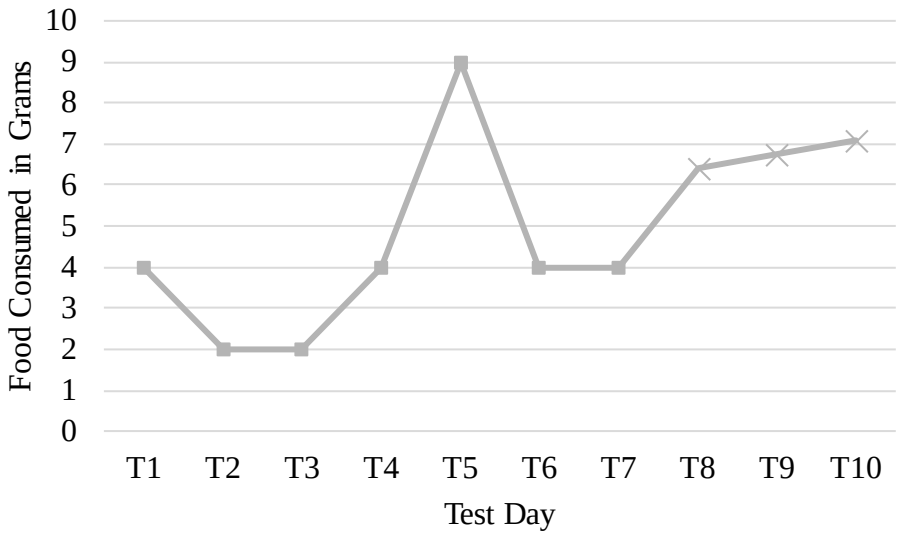


Figure 54 – Rat 29 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 29 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

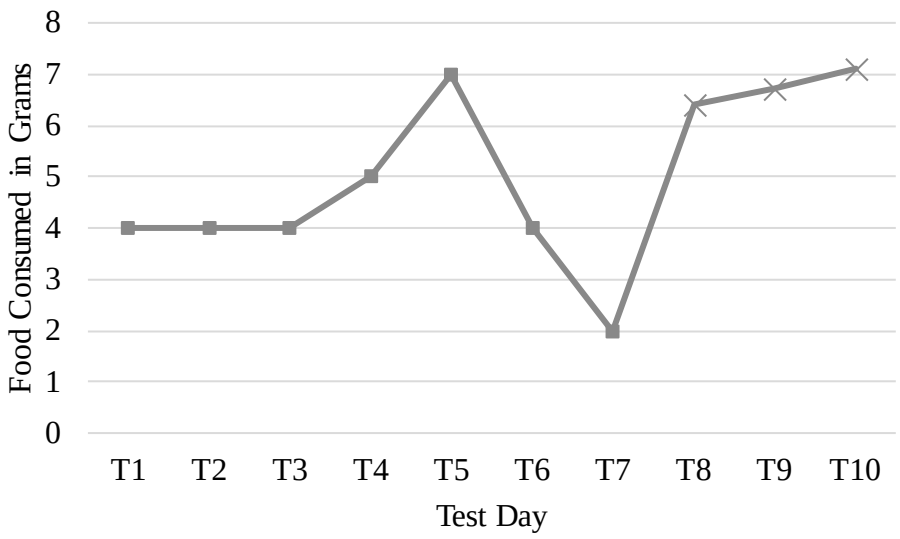


Figure 55 – Rat 30 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 30 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

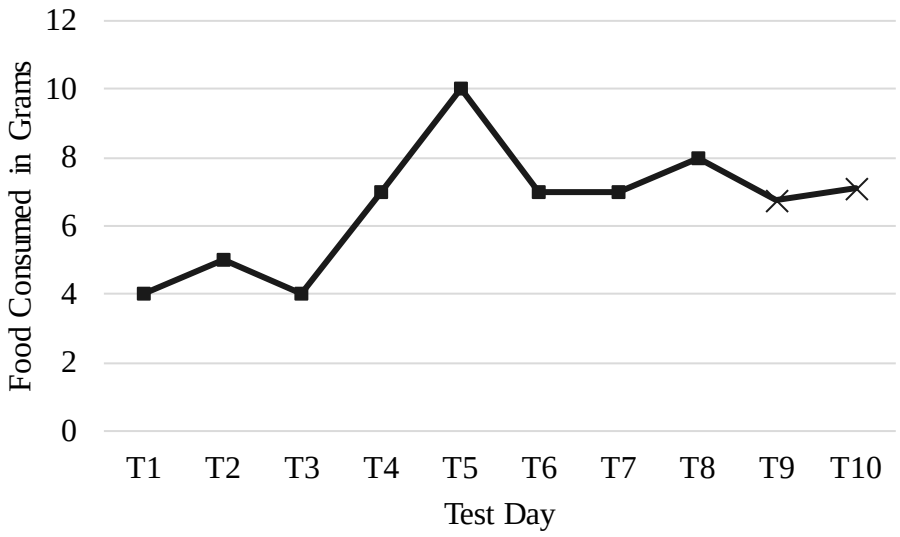


Figure 56 – Rat 31 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 31 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

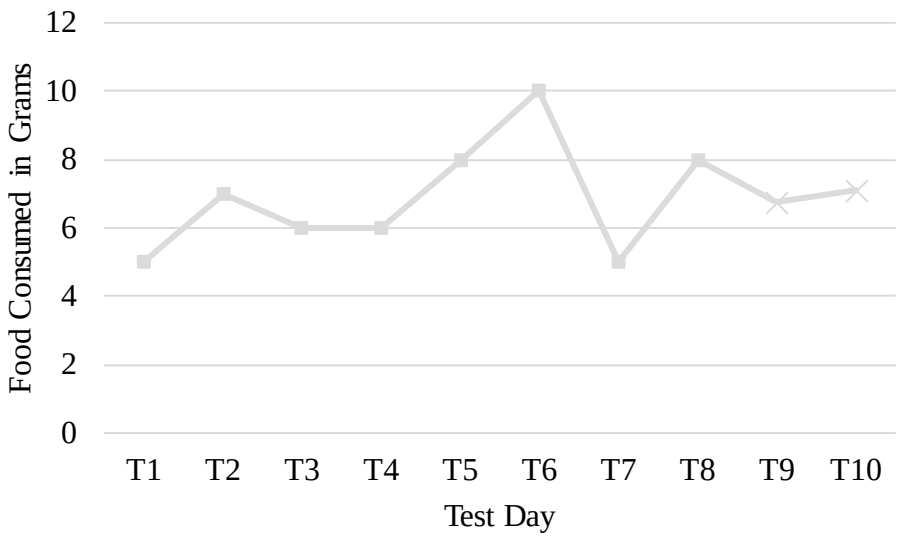


Figure 57 – Rat 32 Food Consumption with Extrapolated Data – This figure shows the daily food consumption of Rat 32 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 6 for the regression line used to determine the missing data.

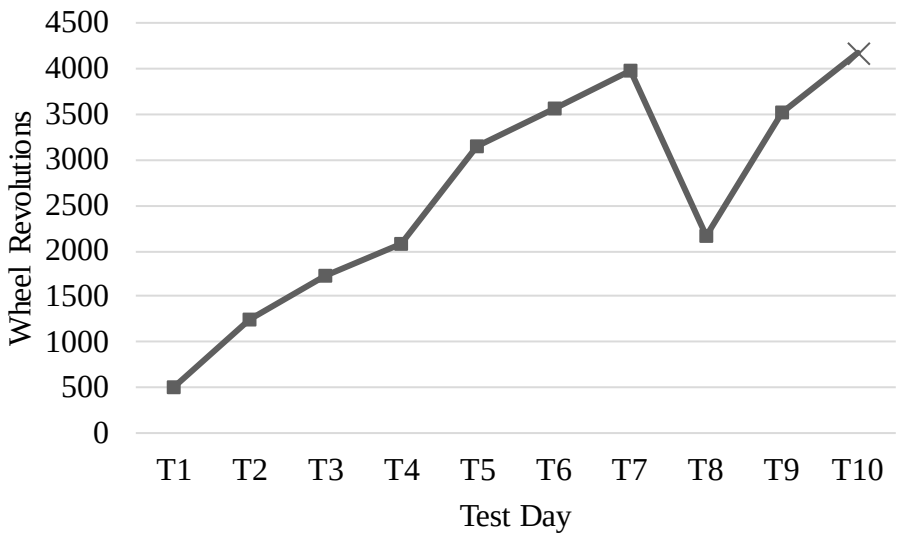


Figure 58 – Rat 5 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 5 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

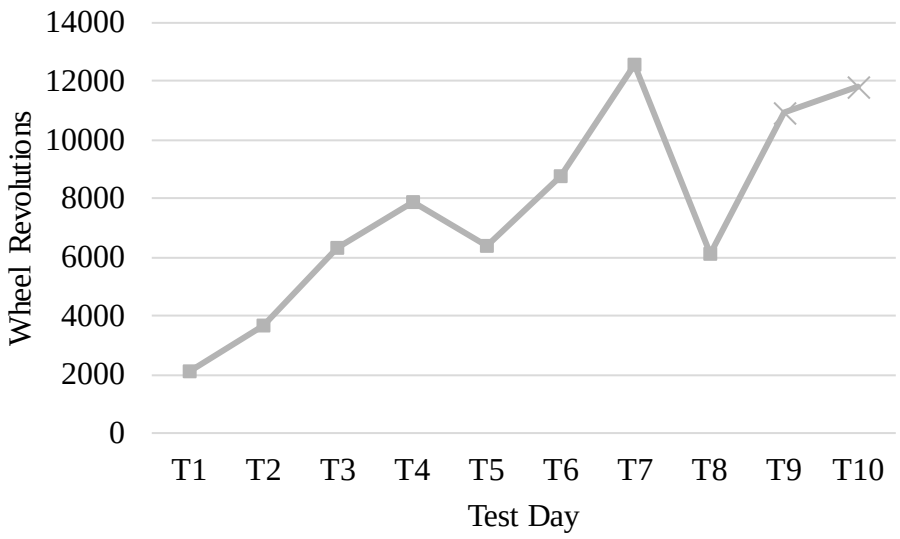


Figure 59 – Rat 7 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 7 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

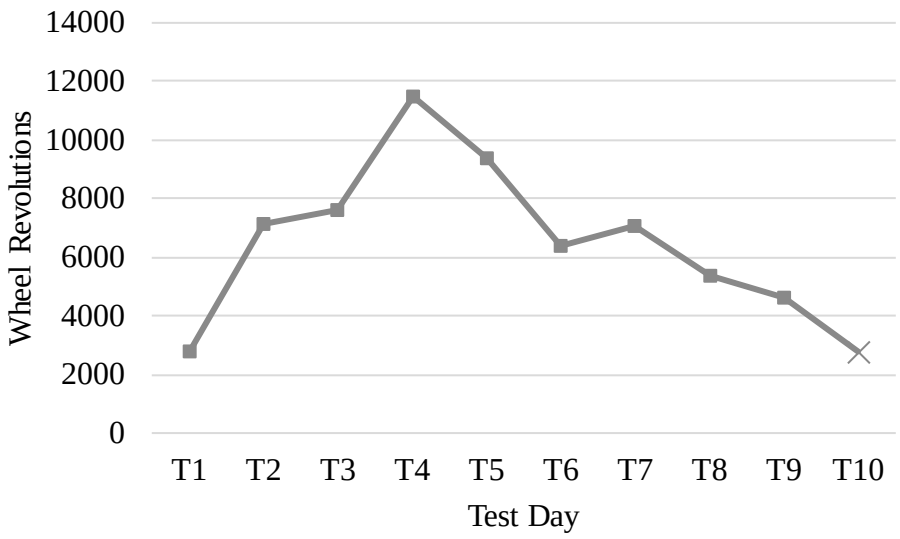


Figure 60 – Rat 13 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 13 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

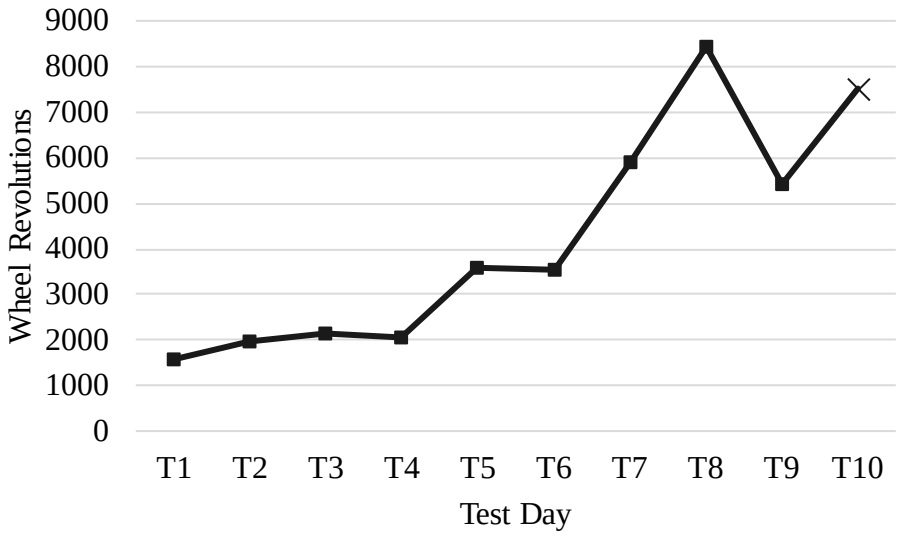


Figure 61 – Rat 17 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 17 with extrapolated data. The extrapolated points are indicated with an “X” on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

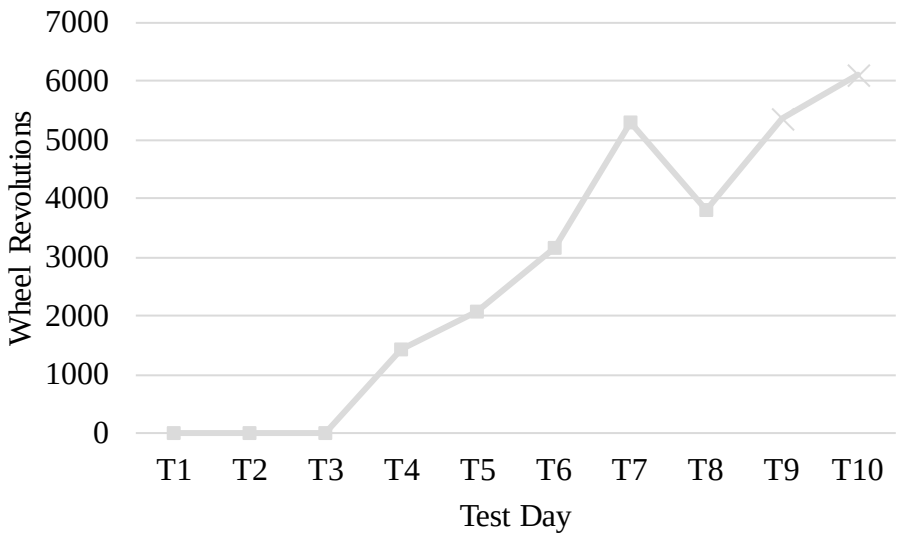


Figure 62 – Rat 25 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 25 with extrapolated data. The extrapolated points are indicated with an “X” on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

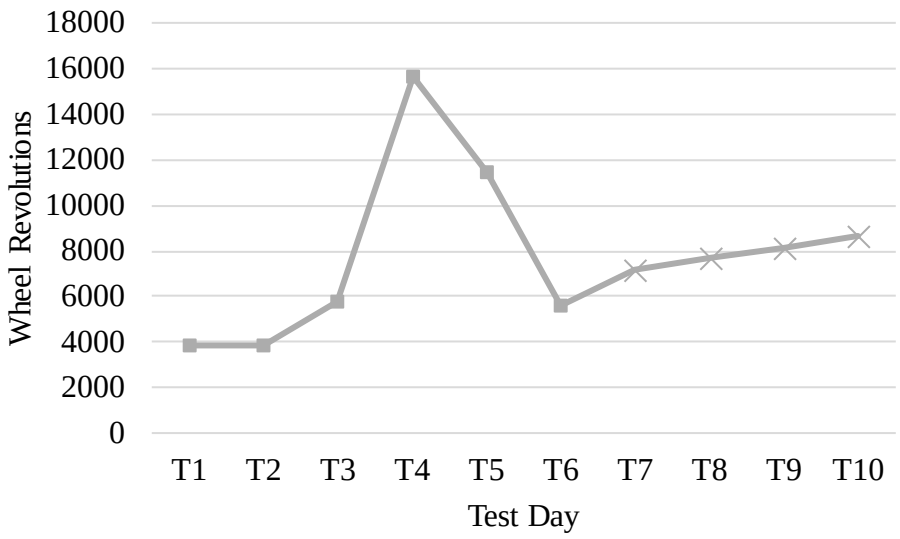


Figure 63 – Rat 26 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 26 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 8 for the regression line used to determine the missing data.

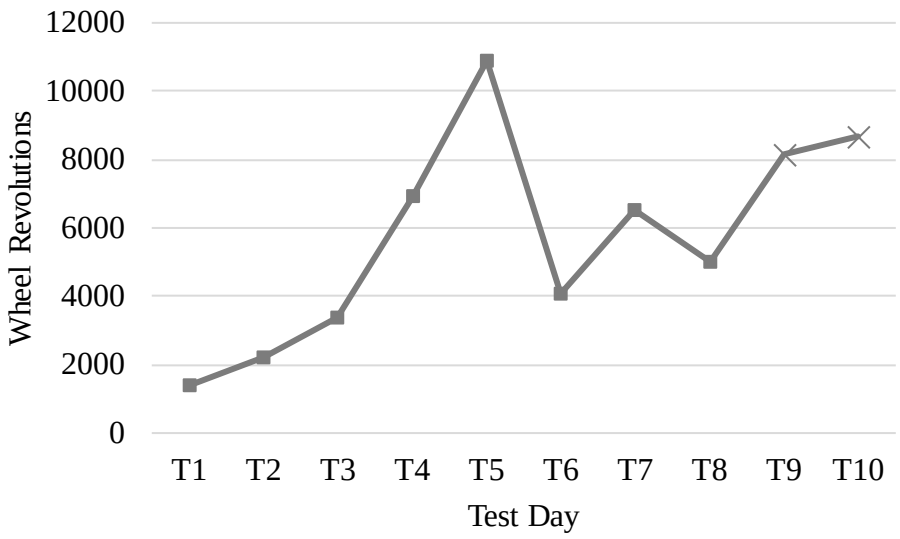


Figure 64 – Rat 27 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 27 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 8 for the regression line used to determine the missing data.

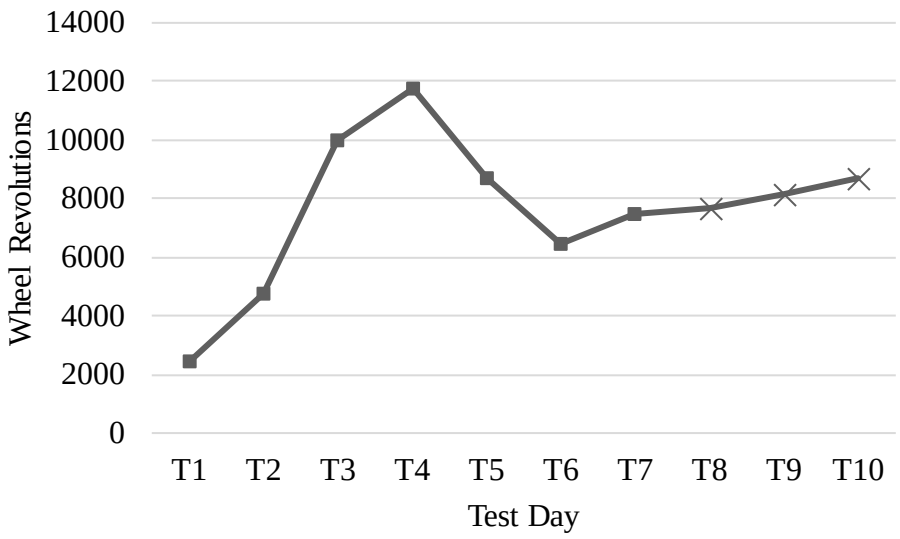


Figure 65 – Rat 28 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 28 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 8 for the regression line used to determine the missing data.

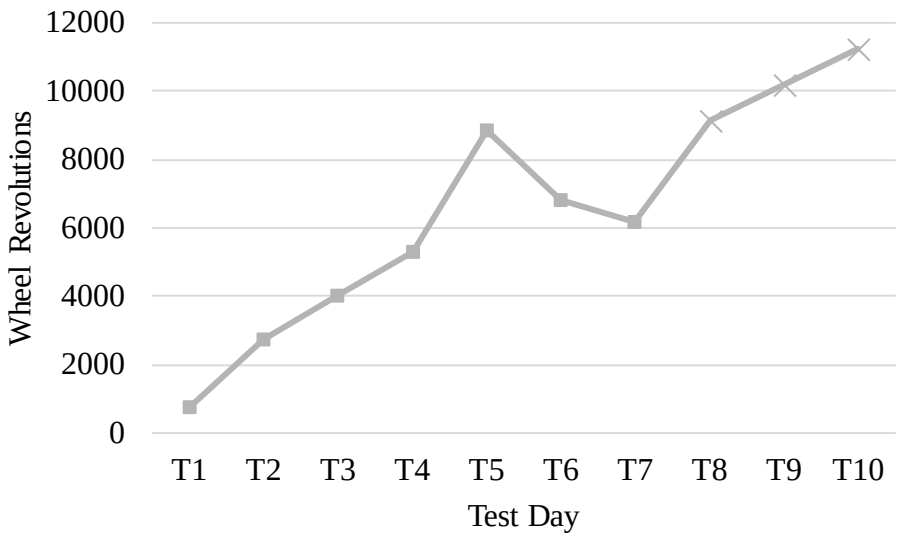


Figure 66 – Rat 29 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 29 with extrapolated data. The extrapolated points are indicated with an “X” on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

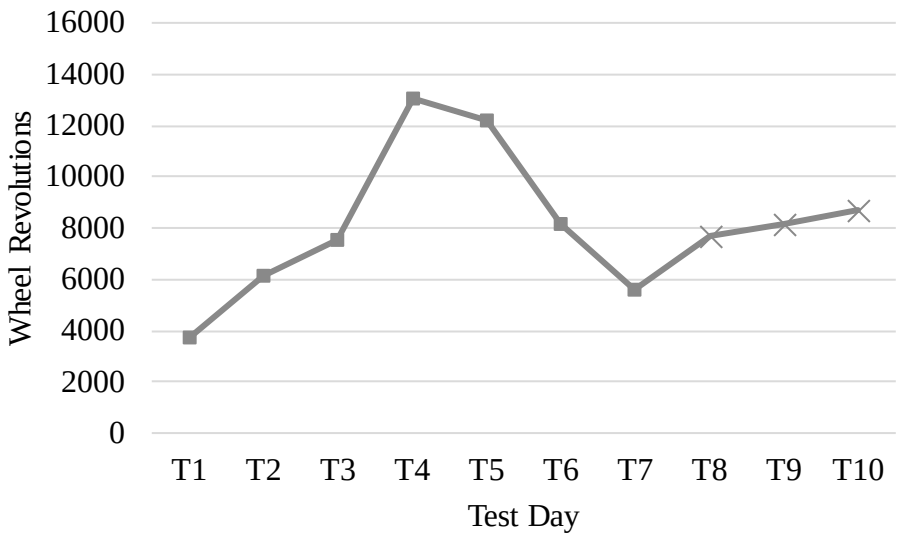


Figure 67 – Rat 30 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 30 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 8 for the regression line used to determine the missing data.

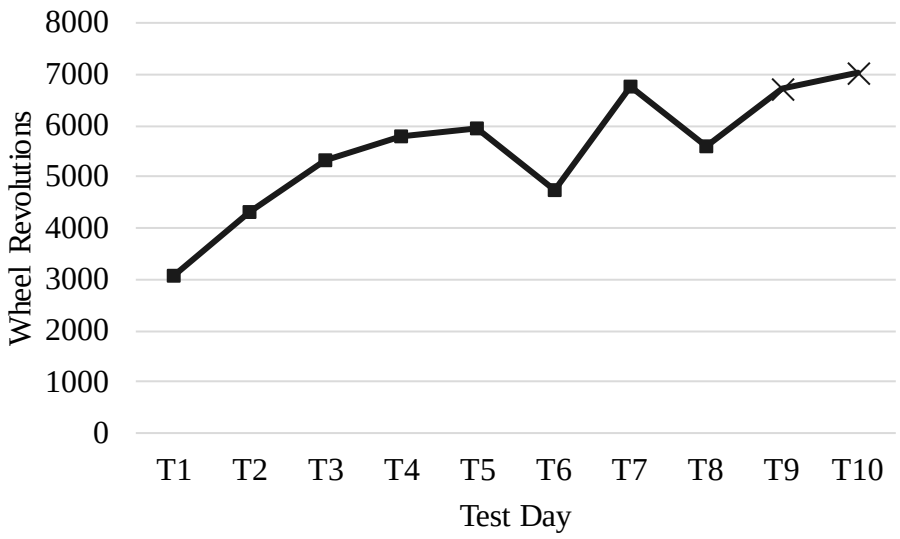


Figure 68 – Rat 31 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 31 with extrapolated data. The extrapolated points are indicated with an "X" on the line graph. Refer to Table 7 for the regression line used to determine the missing data.

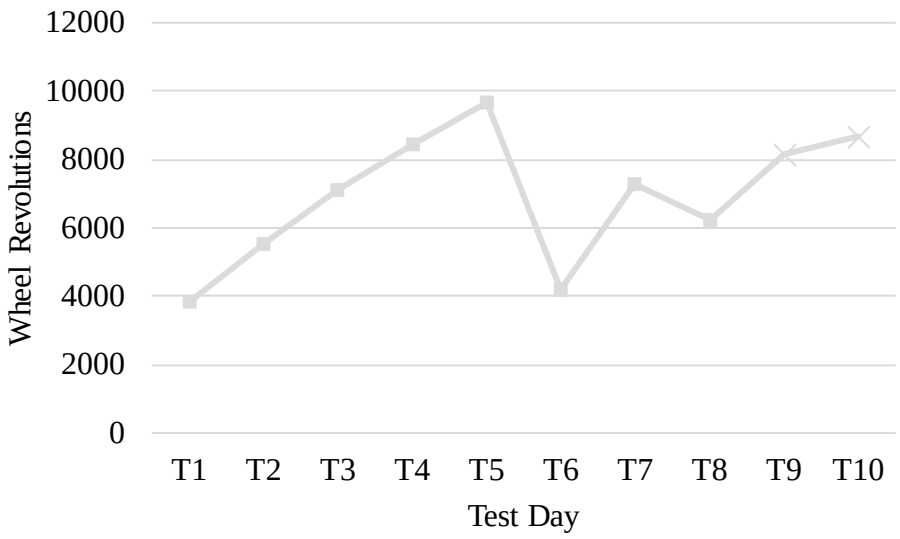


Figure 69 – Rat 32 Wheel Running with Extrapolated Data – This figure shows the daily wheel running of Rat 32 with extrapolated data. The extrapolated points are indicated with an “X” on the line graph. Refer to Table 8 for the regression line used to determine the missing data.

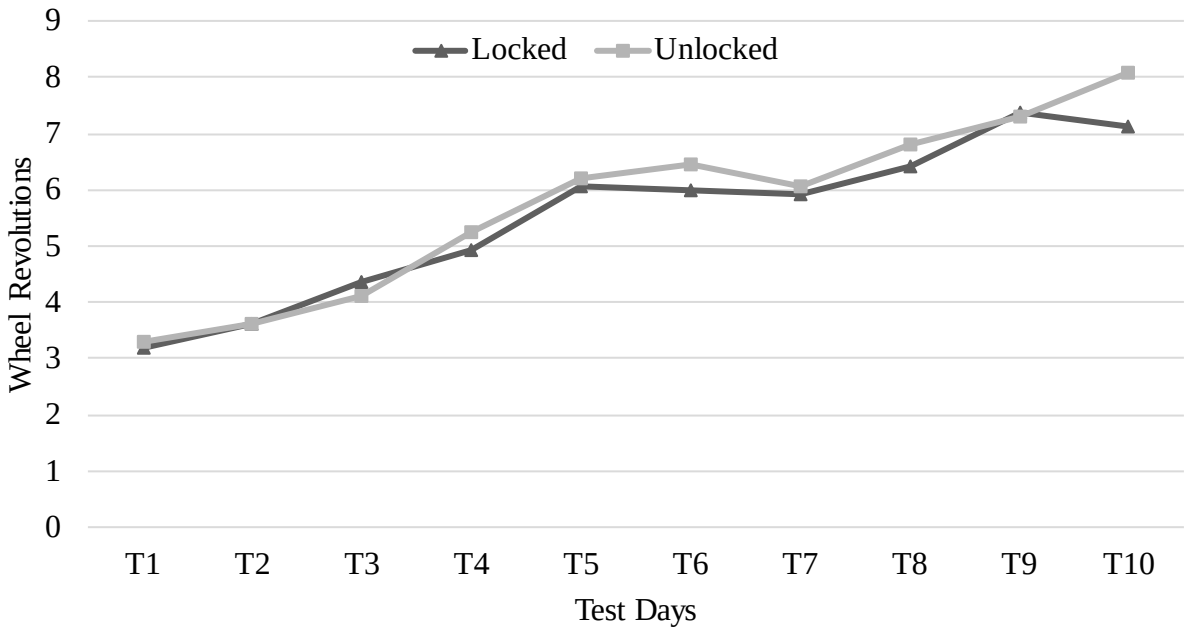


Figure 70 – Food Consumption by Test Day for Locked and Unlocked Groups with Extrapolated Data – This figure shows the comparison of Locked and Unlocked groups on food consumed including extrapolated data. No main effect of Lock ($F(1, 15) = 0.82$, $p = 0.38$) or the interaction of Lock X Days ($F(9, 135) = 0.49$, $p = 0.88$) was found. A main effect of Days ($F(9, 135) = 32.25$, $p < 0.001$, *partial* $\eta^2 = 0.68$) was found.

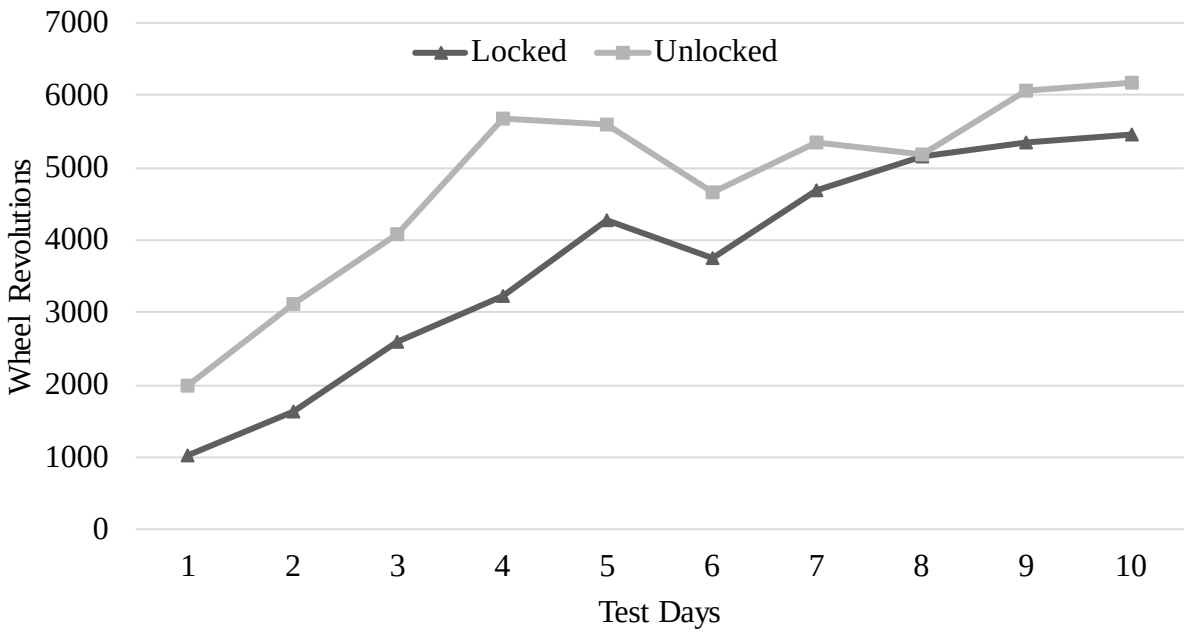


Figure 71 – Wheel Running by Test Day for Locked and Unlocked Groups with Extrapolated Data – This figure shows the comparison of Locked and Unlocked groups on food consumed including extrapolated data. No interaction effect of Lock X Days ($F(9, 135) = 1.82$, $p = 0.07$) was found. A main effect of Days ($F(9, 135) = 20.29$, $p < 0.001$, *partial* $\eta^2 = 0.58$) and Lock ($F(1, 15) = 4.85$, $p = 0.04$, *partial* $\eta^2 = 0.24$) was found.

BIOGRAPHICAL SKETCH

Rolando Alfredo Botello is the youngest son of three sons to Pedro and Rosa Maria Botello. After being born in San Antonio, TX, the family relocated to San Juan, TX. Rolando graduated from Pharr-San Juan-Alamo High School in San Juan, TX as the 6th ranked student in his graduating class of 2012. He graduated with numerous accolades and accumulated over 60 hours of college credit from South Texas College and the University of Texas-Pan American. He attended St. Edward's University in Austin, TX where he graduated with a Bachelor of Arts in Psychology in December of 2014. He continued his graduate studies at the University of Texas Rio Grande Valley and graduated with a Master of Arts in Clinical Psychology in May of 2018. He plans to seek doctoral studies in clinical psychology.

Rolando has been involved with many organizations. During his undergraduate career, he interned at LifeSteps of Round Rock, TX as a substance abuse prevention advocate and family monitor. During graduate studies, he interned at Family Focus Psychological Services under the supervision of Dr. Mireles and Dr. Perez. He also co-coordinated a behavioral neuroscience research experience camp for local McAllen middle school students. Lastly, he served as a graduate and research assistant to Dr. Ernst, Dr. Talavera-Garza, and Dr. Winkel.

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